	L #	Hits	Search Text	DBs	Time Stamp
1	L1	21	vitro near4 sialyla$	USPAT; US-PGPUB	2003/12/05 14:52
2	L2	27	(commercial or scale or batch) near4 sialyl$	USPAT; US-PGPUB	2003/12/05 14:53

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	21	vitro near4 sialyla$	USPAT; US-PGPUB	2003/12/05 12:36

PGPUB-DOCUMENT-NUMBER: 20030180835

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030180835 A1

TITLE: In vitro modification of glycosylation patterns of recombinant glycopeptides

PUBLICATION-DATE: September 25, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bayer, Robert J.	San Diego	CA	US	

APPL-NO: 10/ 391035

DATE FILED: March 17, 2003

RELATED-US-APPL-DATA:

child 10391035 A1 20030317

parent continuation-of 09855320 20010514 US PENDING

non-provisional-of-provisional 60203851 20000512 US

US-CL-CURRENT: 435/68.1, 435/193 , 530/395

ABSTRACT:

This invention provides methods for modifying glycosylation patterns of glycopeptides, including recombinantly produced glycopeptides. Also provided are glycopeptide compositions in which the glycopeptides have a uniform glycosylation pattern.

---------- KWIC ---------

Detail Description Paragraph - DETX (73):
[0121] Thus, in some embodiments, the invention provides methods for in **vitro sialylation** of saccharide groups present on a glycopeptide that first involves modifying the glycopeptide to create a suitable acceptor. Examples of preferred methods of multi-enzyme synthesis of desired oligosaccharide determinants are as follows.

Detail Description Paragraph - DETX (128):
[0175] The present examples exemplify the methods of the invention. Example 1 sets forth the introduction of sialyl Lewis x structures onto a peptide using

sialylation and fucosylation in vitro. Example 2 sets forth the results of an investigation into the substrated specificity and fucosylation activity of two fucosyltransferases, FucT-V and FucT-VI. Example 3 sets forth an exemplary fucosylation process of the invention utilizing the protein RsCD4 as a substrate for fucosylation. The fucosylation step is preceded by a sialylation step. Example 4 sets forth an exemplar assay for determining the ability of a fucosyltransferase to act on a particular glycoprotein.

Detail Description Paragraph - DETX (130):

[0176] Example 1 sets forth the introduction of sialyl Lewis x structures onto a peptide using using **sialylation and fucosylation in vitro**.

PGPUB-DOCUMENT-NUMBER: 20030124645

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030124645 A1

TITLE: Practical in **vitro sialylation** of recombinant glycoproteins

PUBLICATION-DATE: July 3, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Paulson, James C.	Del Mar	CA	US	
Bayer, Robert J.	San Diego	CA	US	
Sjoberg, Eric	San Diego	CA	US	

APPL-NO: 10/ 219120

DATE FILED: August 13, 2002

RELATED-US-APPL-DATA:

child 10219120 A1 20020813

parent continuation-of 10007331 20011109 US PENDING

child 10007331 20011109 US

parent division-of 09007741 19980115 US GRANTED

parent-patent 6399336 US

non-provisional-of-provisional 60035710 19970116 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
WO	PCT/US98/00835	1998WO-PCT/US98/00835	January 15, 1998

US-CL-CURRENT: 435/68.1, 536/53

ABSTRACT:

This invention provides methods for practical in **vitro sialylation** of glycoproteins, including recombinantly produced glycoproteins. The methods are useful for large-scale modification of sialylation patterns.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 60/035,710, filed Jan. 16, 1997, which is incorporated herein by reference in

its entirety for all purposes.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):
This invention provides methods for practical in **vitro sialylation** of glycoproteins, including recombinantly produced glycoproteins. The methods are useful for large-scale modification of sialylation patterns.

Title - TTL (1):
Practical in **vitro sialylation** of recombinant glycoproteins

Summary of Invention Paragraph - BSTX (3):
[0003] This invention pertains to the field of in **vitro sialylation** of glycoproteins, including recombinant glycoproteins.

Summary of Invention Paragraph - BSTX (10):
[0009] The present invention provides methods for in **vitro sialylation** of saccharide groups present on a recombinantly produced glycoprotein. The methods comprise contacting the saccharide groups with a sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase activity for a sufficient time and under appropriate conditions to transfer sialic acid from the sialic acid donor moiety to said saccharide group.

Detail Description Paragraph - DETX (14):
[0027] The present invention provides methods for efficient in **vitro sialylation** of saccharide groups attached to glycoproteins, in particular recombinantly produced glycoproteins. For example, the methods of the invention are useful for sialylation of recombinantly produced therapeutic glycoproteins that are incompletely sialylated during production in mammalian cells or transgenic animals. The methods involve contacting the saccharide groups with a sialyltransferase and a sialic acid donor moiety for a sufficient time and under appropriate reaction conditions to transfer sialic acid from the sialic acid donor moiety to the saccharide groups. Sialyltransferases comprise a family of glycosyltransferases that transfer sialic acid from the donor substrate CMP-sialic acid to acceptor oligosaccharide substrates. In preferred embodiments, the sialyltransferases used in the methods of the invention are recombinantly produced.

Detail Description Paragraph - DETX (18):
[0031] The in **vitro sialylation** methods provided by the invention are, unlike previously described sialylation methods, practical for commercial-scale production of modified glycoproteins. Thus, the claimed methods provide a practical means for large-scale preparation of glycoproteins having altered sialylation patterns. The methods are well suited for therapeutic glycoproteins that are incompletely sialylated during production in mammalian

cells or transgenic animals. The processes provide an increased and consistent level of terminal sialylation of a glycoprotein.

Detail Description Paragraph - DETX (30):

[0043] Thus, in one embodiment, the invention provides methods for in **vitro sialylation** of saccharide groups present on a glycoprotein that first involves modifying the glycoprotein to create a suitable acceptor. A preferred method for synthesizing an acceptor involves use of a galactosyltransferase. The steps for these methods include:

Claims Text - CLTX (33):

32. A method for in **vitro sialylation** of saccharide groups present on a glycoprotein, said method comprising contacting said saccharide groups with a sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase activity for a sufficient time and under appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said saccharide group, wherein said sialyltransferase is present at a concentration about 50 mU per mg of glycoprotein or less.

Claims Text - CLTX (58):

57. A method for in **vitro sialylation** of saccharide groups present on a glycoprotein, the method comprising contacting the saccharide groups with an ST3Gal III sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase activity for a sufficient time and under conditions to transfer sialic acid from said sialic acid donor moiety to said saccharide group, wherein said ST3Gal III sialyltransferase is present at a concentration of less than about 50 mU per mg of glycoprotein.

PGPUB-DOCUMENT-NUMBER: 20030120045

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030120045 A1

TITLE: Erythropoietin conjugates

PUBLICATION-DATE: June 26, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bailon, Pascal Sebastian	Florham Park	NJ	US	

APPL-NO: 10/ 293551

DATE FILED: November 14, 2002

RELATED-US-APPL-DATA:

child 10293551 A1 20021114

parent continuation-of 09604938 20000627 US PENDING

non-provisional-of-provisional 60142254 19990702 US

non-provisional-of-provisional 60150225 19990823 US

non-provisional-of-provisional 60151548 19990831 US

non-provisional-of-provisional 60166151 19991117 US

US-CL-CURRENT: 530/397

ABSTRACT:

Conjugates of erythropoietin with poly(ethylene glycol) comprise an erythropoietin glycoprotein having at least one free amino group and having the in vivo biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells and selected from the group consisting of human erythropoietin and analogs thereof which have sequence of human erythropoietin modified by the addition of from 1 to 6 glycosylation sites or a rearrangement of at least one glycosylation site; said glycoprotein being covalently linked to "n" poly(ethylene glycol) groups of the formula --CO--(CH.sub.2).sub.x(OCH.su- b.2CH.sub.2).sub.m--OR with the carbonyl of each poly(ethylene glycol) group forming an amide bond with one of said amino groups; wherein R is lower alkyl; x is 2 or 3; m is about 450 to about 900; n is from 1 to 3; and n and m are chosen so that the molecular weight of the conjugate minus the erythropoietin glycoprotein is from 20 kilodaltons to 100 kilodaltons.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The priority of U.S. Provisional Application No. 60/142,254, filed Jul. 2, 1999; No. 60/150,225, filed Aug. 23, 1999; No. 60/151,548, filed Aug. 31, 1999; No. 60/166,151, filed Nov. 17, 1999 and U.S. application Ser. No. 09/604,938, filed Jun. 27, 2000 is claimed.

---------- KWIC ---------

Detail Description Paragraph - DETX (11):

[0021] In an embodiment, P may be the residue of a glycoprotein analog having from 1 to 6 additional sites for glycosylation. Glycosylation of a protein, with one or more oligosaccharide groups, occurs at specific locations along a polypeptide backbone and greatly affects the physical properties of the protein such as protein stability, secretion, subcellular localization, and biological activity. Glycosylation is usually of two types. O-linked oligosaccharides are attached to serine or threonine residues and N-linked oligosaccharides are attached to asparagine residues. One type of oligosaccharide found on both N-linked and O-linked oligosaccharides is N-acetylneuraminic acid (sialic acid), which is a family of amino sugars containing 9 or more carbon atoms. Sialic acid is usually the terminal residue on both N-linked and O-linked oligosaccharides and, because it bears a negative charge, confers acidic properties to the glycoprotein. Human erythropoietin, having 165 amino acids, contains three N-linked and one O-linked oligosaccharide chains which comprise about 40% of the total molecular weight of the glycoprotein. N-linked glycosylation occurs at asparagine residues located at positions 24, 38, and 83 and O-linked glycosylation occurs at a serine residue located at position 126. The oligosaccharide chains are modified with terminal sialic acid residues. Enzymatic removal of all sialic acid residues from the glycosylated erythropoietin results in loss of in vivo activity but not in **vitro activity because sialylation** of erythropoietin prevents its binding, and subsequent clearance, by hepatic binding protein.

PGPUB-DOCUMENT-NUMBER: 20030113343

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030113343 A1

TITLE: Identification and characterization of novel pneumococcal choline binding protein, CbpG, and diagnostic and therapeutic uses thereof

PUBLICATION-DATE: June 19, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Tuomanen, Elaine I.	Germantown	TN	US	
Gosink, Khoosheh	Cordova	TN	US	
Masure, Robert	Germantown	TN	US	

APPL-NO: 10/ 243977

DATE FILED: September 13, 2002

RELATED-US-APPL-DATA:

child 10243977 A1 20020913

parent continuation-of 09287070 19990406 US GRANTED

parent-patent 6495139 US

child 09287070 19990406 US

parent continuation-in-part-of 09196389 19981119 US ABANDONED

US-CL-CURRENT: 424/190.1, 435/252.3 , 435/320.1 , 435/6 , 435/69.3 , 435/7.32 , 514/44 , 530/350 , 536/23.7

ABSTRACT:

The present invention provides isolated polypeptides comprising an amino acid sequence of a choline binding protein CbpG. This invention provides an isolated polypeptide comprising an amino acid sequence of a choline binding polypeptide CbpG or N-terminal CbpG truncate, including analogs, variants, mutants, derivatives and fragments thereof. This invention further provides an isolated immunogenic polypeptide comprising an amino acid sequence of a choline binding protein CbpG. This invention provides an isolated nucleic acid encoding a polypeptide comprising an amino acid sequence of a choline binding protein CbpG. This invention provides pharmaceutical compositions, vaccines, and diagnostic and therapeutic methods of use of the isolated polypeptides and nucleic acids. Assays for compounds which alter or inactivate the polypeptides of the present invention for use in therapy are also provided.

RELATED APPLICATIONS

[0001] The present application is a continuation-in-part of copending application Ser. No. 09/196,389 filed Nov. 19, 1998, of which the instant application claims the benefit of the filing date pursuant to 35 U.S.C. .sctn.120, and which is incorporated herein by reference in its entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (166):

[0210] We have tested the ability of this mutant to adhere to human Detroit cells, and to carbohydrates in vitro. The in vitro adhesion experiments were done as previously described (Cundell D. R. et al (1995) Infect Immun 63(3):757-761) and the data shown in Table 2 indicate that there is a 40% reduction in adhesion to Detroit human nasopharyngeal cell line by the CbpG-deficient mutant and a 80-90% reduction in adhesion to both lacto-N-neotrose (LNnT) and **sialylactose in vitro**. These data along with the in vivo colonization data suggest that CbpG plays a role in adherence to cells of the nasopharynx and that the binding may occur through sugars on the eukaryotic cells.

PGPUB-DOCUMENT-NUMBER: 20030092160

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030092160 A1

TITLE: Recombinant protein production in a human cell

PUBLICATION-DATE: May 15, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bout, Abraham	Moerkapelle		NL	
Hateboer, Guus	Heemstede		NL	
Verhulst, Karina Cornelia	Leiden		NL	
Uytdehaag, Alphonsus Gerardus	Debilt		NL	
Schouten, Govert Johan	Leiderdorp		NL	

APPL-NO: 10/ 234007

DATE FILED: September 3, 2002

RELATED-US-APPL-DATA:

child 10234007 A1 20020903

parent division-of 09549463 20000414 US PENDING

non-provisional-of-provisional 60129452 19990415 US

US-CL-CURRENT: 435/235.1, 435/325 , 435/456

ABSTRACT:

Methods and compositions for the production of recombinant proteins in a human cell line. The methods and compositions are particularly useful for generating stable expression of human recombinant proteins of interest that are modified post-translationally, for example, by glycosylation. Such proteins may have advantageous properties in comparison with their counterparts produced in non-human systems such as Chinese hamster ovary cells.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of application Ser. No. 09/549,463, filed Apr. 14, 2002, pending, the contents of the entirety of which, including its sequence listing, is incorporated by this reference, which application claims priority under 35 U.S.C. .sctn. 119(e) to Provisional Patent Application Serial No. 60/129,452 filed Apr. 15, 1999.

---------- KWIC ---------

Detail Description Paragraph - DETX (158):

[0161] The function of recombinant EPO in vivo is determined by its half-life in the bloodstream. Removal of EPO takes place by liver enzymes that bind to galactose residues in the glycans that are not protected by sialic acids and by removal through the kidney. Whether this filtering by the kidney is due to misfolding or due to under- or mis-glycosylation is unknown. Furthermore, EPO molecules that reach their targets in the bone marrow and bind to the EPO receptor on progenitor cells are also removed from circulation. Binding to the EPO receptor and down stream signalling depends heavily on a proper glycosylation status of the EPO molecule. Sialic acids can, to some extent, inhibit binding of EPO to the EPO receptor, resulting in a lower effectivity of the protein. However, since the sialic acids prevent EPO from removal, these sugars are essential for its function to protect the protein on its travel to the EPO receptor. When sialic acids are removed from EPO in vitro, a better binding to the receptor occurs, resulting in a stronger down stream signalling. This means that the functionalities in vivo and in vitro are significantly different, although a proper EPO receptor binding property can be checked in **vitro despite the possibility of an under-sialylation** causing a short half-life in vivo (Takeuchi et al. 1989).

PGPUB-DOCUMENT-NUMBER: 20030040037

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030040037 A1

TITLE: In vitro modification of glycosylation patterns of recombinant glycopeptides

PUBLICATION-DATE: February 27, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bayer, Robert J.	San Diego	CA	US	

APPL-NO: 10/ 219197

DATE FILED: August 13, 2002

RELATED-US-APPL-DATA:

child 10219197 A1 20020813

parent continuation-of 09855320 20010514 US PENDING

non-provisional-of-provisional 60203851 20000512 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
WO	PCT/US01/15693	2001WO-PCT/US01/15693	May 14, 2001

US-CL-CURRENT: 435/68.1, 435/193 , 435/252.3 , 435/69.1

ABSTRACT:

This invention provides methods for modifying glycosylation patterns of glycopeptides, including recombinantly produced glycopeptides. Also provided are glycopeptide compositions in which the glycopeptides have a uniform glycosylation pattern.

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of prior U.S. provisional application No. 60/203,851, filed May 12, 2000.

---------- KWIC ---------

Detail Description Paragraph - DETX (73):
[0121] Thus, in some embodiments, the invention provides methods for in

vitro sialylation of saccharide groups present on a glycopeptide that first involves modifying the glycopeptide to create a suitable acceptor. Examples of preferred methods of multi-enzyme synthesis of desired oligosaccharide determinants are as follows.

Detail Description Paragraph - DETX (128):

[0175] The present examples exemplify the methods of the invention. Example 1 sets forth the introduction of sialyl Lewis x structures onto a peptide using **sialylation and fucosylation in vitro**. Example 2 sets forth the results of an investigation into the substrated specificity and fucosylation activity of two fucosyltransferases, FucT-V and FucT-VI. Example 3 sets forth an exemplary fucosylation process of the invention utilizing the protein RsCD4 as a substrate for fucosylation. The fucosylation step is preceded by a sialylation step. Example 4 sets forth an exemplar assay for determining the ability of a fucosyltransferase to act on a particular glycoprotein.

Detail Description Paragraph - DETX (130):

[0176] Example 1 sets forth the introduction of sialyl Lewis x structures onto a peptide using using **sialylation and fucosylation in vitro**.

PGPUB-DOCUMENT-NUMBER: 20030003529

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030003529 A1

TITLE: Vitro modification of glycosylation patterns of recombinant glycopeptides

PUBLICATION-DATE: January 2, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bayer, Robert J.	San Diego	CA	US	

APPL-NO: 10/ 198806

DATE FILED: July 19, 2002

RELATED-US-APPL-DATA:

child 10198806 A1 20020719

parent division-of 09855320 20010514 US PENDING

non-provisional-of-provisional 60203851 20000512 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
	PCT/US01/15693		May 14, 2001

US-CL-CURRENT: 435/68.1, 435/193 , 435/69.1 , 530/322

ABSTRACT:

This invention provides methods for modifying glycosylation patterns of glycopeptides, including recombinantly produced glycopeptides. Also provided are glycopeptide compositions in which the glycopeptides have a uniform glycosylation pattern.

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of prior U.S. provisional application No. 60/203,851, filed May 12, 2000.

---------- KWIC ---------

Detail Description Paragraph - DETX (73):
[0121] Thus, in some embodiments, the invention provides methods for in

vitro sialylation of saccharide groups present on a glycopeptide that first involves modifying the glycopeptide to create a suitable acceptor. Examples of preferred methods of multi-enzyme synthesis of desired oligosaccharide determinants are as follows.

Detail Description Paragraph - DETX (129):

[0176] The present examples exemplify the methods of the invention. Example 1 sets forth the introduction of sialyl Lewis x structures onto a peptide using **sialylation and fucosylation in vitro**. Example 2 sets forth the results of an investigation into the substrated specificity and fucosylation activity of two fucosyltransferases, FucT-V and FucT-VI. Example 3 sets forth an exemplary fucosylation process of the invention utilizing the protein RsCD4 as a substrate for fucosylation. The fucosylation step is preceded by a sialylation step. Example 4 sets forth an exemplar assay for determining the ability of a fucosyltransferase to act on a particular glycoprotein.

Detail Description Paragraph - DETX (131):

[0177] Example 1 sets forth the introduction of sialyl Lewis x structures onto a peptide using using **sialylation and fucosylation in vitro**.

PGPUB-DOCUMENT-NUMBER: 20020160460

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020160460 A1

TITLE: Practical in **vitro sialylation** of recombinant glycoproteins

PUBLICATION-DATE: October 31, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Paulson, James C.	Del Mar	CA	US	
Bayer, Robert J.	San Diego	CA	US	
Sjoberg, Eric	San Diego	CA	US	

APPL-NO: 10/ 081456

DATE FILED: February 21, 2002

RELATED-US-APPL-DATA:

child 10081456 A1 20020221

parent continuation-of 09007741 19980115 US GRANTED

parent-patent 6399336 US

non-provisional-of-provisional 60035710 19970116 US

US-CL-CURRENT: 435/84, 435/193 , 536/53

ABSTRACT:

This invention provides methods for practical in **vitro sialylation** of glycoproteins, including recombinantly produced glycoproteins. The methods are useful for large-scale modification of sialylation patterns.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application 60/035,710, filed Jan. 16, 1997, which is incorporated herein by reference in its entirety for all purposes.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):
This invention provides methods for practical in **vitro sialylation** of

glycoproteins, including recombinantly produced glycoproteins. The methods are useful for large-scale modification of sialylation patterns.

Title - TTL (1):
Practical in **vitro sialylation** of recombinant glycoproteins

Summary of Invention Paragraph - BSTX (3):
[0003] This invention pertains to the field of in **vitro sialylation** of glycoproteins, including recombinant glycoproteins.

Summary of Invention Paragraph - BSTX (10):
[0009] The present invention provides methods for in **vitro sialylation** of saccharide groups present on a recombinantly produced glycoprotein. The methods comprise contacting the saccharide groups with a sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase activity for a sufficient time and under appropriate conditions to transfer sialic acid from the sialic acid donor moiety to said saccharide group.

Detail Description Paragraph - DETX (23):
[0034] The present invention provides methods for efficient in **vitro sialylation** of saccharide groups attached to glycoproteins, in particular recombinantly produced glycoproteins. For example, the methods of the invention are useful for sialylation of recombinantly produced therapeutic glycoproteins that are incompletely sialylated during production in mammalian cells or transgenic animals. The methods involve contacting the saccharide groups with a sialyltransferase and a sialic acid donor moiety for a sufficient time and under appropriate reaction conditions to transfer sialic acid from the sialic acid donor moiety to the saccharide groups. Sialyltransferases comprise a family of glycosyltransferases that transfer sialic acid from the donor substrate CMP-sialic acid to acceptor oligosaccharide substrates. In preferred embodiments, the sialyltransferases used in the methods of the invention are recombinantly produced.

Detail Description Paragraph - DETX (27):
[0038] The in **vitro sialylation** methods provided by the invention are, unlike previously described sialylation methods, practical for commercial-scale production of modified glycoproteins. Thus, the claimed methods provide a practical means for large-scale preparation of glycoproteins having altered sialylation patterns. The methods are well suited for therapeutic glycoproteins that are incompletely sialylated during production in mammalian cells or transgenic animals. The processes provide an increased and consistent level of terminal sialylation of a glycoprotein.

Detail Description Paragraph - DETX (39):
[0050] Thus, in one embodiment, the invention provides methods for in **vitro sialylation** of saccharide groups present on a glycoprotein that first involves modifying the glycoprotein to create a suitable acceptor. A preferred method

for synthesizing an acceptor involves use of a galactosyltransferase. The steps for these methods include:

Claims Text - CLTX (33):

32. A method for in **vitro sialylation** of saccharide groups present on a glycoprotein, said method comprising contacting said saccharide groups with a sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase activity for a sufficient time and under appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said saccharide group, wherein said sialyltransferase is present at a concentration about 50 mU per mg of glycoprotein or less.

Claims Text - CLTX (58):

57. A method for in **vitro sialylation** of saccharide groups present on a glycoprotein, the method comprising contacting the saccharide groups with an ST3Gal III sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase activity for a sufficient time and under conditions to transfer sialic acid from said sialic acid donor moiety to said saccharide group, wherein said ST3Gal III sialyltransferase is present at a concentration of less than about 50 mU per mg of glycoprotein.

PGPUB-DOCUMENT-NUMBER: 20020142370

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020142370 A1

TITLE: Practical in **vitro sialylation** of recombinant glycoproteins

PUBLICATION-DATE: October 3, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Paulson, James C.	Del Mar	CA	US	
Bayer, Robert J.	San Diego	CA	US	
Sjoberg, Eric	San Diego	CA	US	

APPL-NO: 10/ 081455

DATE FILED: February 21, 2002

RELATED-US-APPL-DATA:

child 10081455 A1 20020221

parent continuation-of 09007741 19980115 US GRANTED

parent-patent 6399336 US

non-provisional-of-provisional 60035710 19970116 US

US-CL-CURRENT: 435/68.1, 435/200 , 536/53

ABSTRACT:

This invention provides methods for practical in **vitro sialylation** of glycoproteins, including recombinantly produced glycoproteins. The methods are useful for large-scale modification of sialylation patterns.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application 60/035,710, filed Jan. 16, 1997, which is incorporated herein by reference in its entirety for all purposes.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):
This invention provides methods for practical in **vitro sialylation** of

glycoproteins, including recombinantly produced glycoproteins. The methods are useful for large-scale modification of sialylation patterns.

Title - TTL (1):
Practical in **vitro sialylation** of recombinant glycoproteins

Summary of Invention Paragraph - BSTX (3):
[0003] This invention pertains to the field of in **vitro sialylation** of glycoproteins, including recombinant glycoproteins.

Summary of Invention Paragraph - BSTX (10):
[0009] The present invention provides methods for in **vitro sialylation** of saccharide groups present on a recombinantly produced glycoprotein. The methods comprise contacting the saccharide groups with a sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase activity for a sufficient time and under appropriate conditions to transfer sialic acid from the sialic acid donor moiety to said saccharide group.

Detail Description Paragraph - DETX (14):
[0026] The present invention provides methods for efficient in **vitro sialylation** of saccharide groups attached to glycoproteins, in particular recombinantly produced glycoproteins. For example, the methods of the invention are useful for sialylation of recombinantly produced therapeutic glycoproteins that are incompletely sialylated during production in mammalian cells or transgenic animals. The methods involve contacting the saccharide groups with a sialyltransferase and a sialic acid donor moiety for a sufficient time and under appropriate reaction conditions to transfer sialic acid from the sialic acid donor moiety to the saccharide groups. Sialyltransferases comprise a family of glycosyltransferases that transfer sialic acid from the donor substrate CMP-sialic acid to acceptor oligosaccharide substrates. In preferred embodiments, the sialyltransferases used in the methods of the invention are recombinantly produced.

Detail Description Paragraph - DETX (18):
[0030] The in **vitro sialylation** methods provided by the invention are, unlike previously described sialylation methods, practical for commercial-scale production of modified glycoproteins. Thus, the claimed methods provide a practical means for large-scale preparation of glycoproteins having altered sialylation patterns. The methods are well suited for therapeutic glycoproteins that are incompletely sialylated during production in mammalian cells or transgenic animals. The processes provide an increased and consistent level of terminal sialylation of a glycoprotein.

Detail Description Paragraph - DETX (30):
[0042] Thus, in one embodiment, the invention provides methods for in **vitro sialylation** of saccharide groups present on a glycoprotein that first involves modifying the glycoprotein to create a suitable acceptor. A preferred method

for synthesizing an acceptor involves use of a galactosyltransferase. The steps for these methods include:

Claims Text - CLTX (33):
32. A method for in **vitro sialylation** of saccharide groups present on a glycoprotein, said method comprising contacting said saccharide groups with a sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase activity for a sufficient time and under appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said saccharide group, wherein said sialyltransferase is present at a concentration about 50 mU per mg of glycoprotein or less.

Claims Text - CLTX (58):
57. A method for in **vitro sialylation** of saccharide groups present on a glycoprotein, the method comprising contacting the saccharide groups with an ST3Gal III sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase activity for a sufficient time and under conditions to transfer sialic acid from said sialic acid donor moiety to said saccharide group, wherein said ST3Gal III sialyltransferase is present at a concentration of less than about 50 mU per mg of glycoprotein.

PGPUB-DOCUMENT-NUMBER: 20020119516

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020119516 A1

TITLE: Practical in **vitro sialylation** of recombinant glycoproteins

PUBLICATION-DATE: August 29, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Paulson, James C.	Del Mar	CA	US	
Bayer, Robert J.	San Diego	CA	US	
Sjoberg, Eric	San Diego	CA	US	

APPL-NO: 10/ 007331

DATE FILED: November 9, 2001

RELATED-US-APPL-DATA:

child 10007331 A1 20011109

parent division-of 09007741 19980115 US PENDING

non-provisional-of-provisional 60035710 19970116 US

US-CL-CURRENT: 435/68.1, 435/193

ABSTRACT:

This invention provides methods for practical in **vitro sialylation** of glycoproteins, including recombinantly produced glycoproteins. The methods are useful for large-scale modification of sialylation patterns.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 60/035,710, filed Jan. 16, 1997, which is incorporated herein by reference in its entirety for all purposes.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):

This invention provides methods for practical in **vitro sialylation** of glycoproteins, including recombinantly produced glycoproteins. The methods are useful for large-scale modification of sialylation patterns.

Title - TTL (1):
Practical in **vitro sialylation** of recombinant glycoproteins

Summary of Invention Paragraph - BSTX (3):
[0003] This invention pertains to the field of in **vitro sialylation** of glycoproteins, including recombinant glycoproteins.

Summary of Invention Paragraph - BSTX (10):
[0009] The present invention provides methods for in **vitro sialylation** of saccharide groups present on a recombinantly produced glycoprotein. The methods comprise contacting the saccharide groups with a sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase activity for a sufficient time and under appropriate conditions to transfer sialic acid from the sialic acid donor moiety to said saccharide group.

Detail Description Paragraph - DETX (23):
[0036] The present invention provides methods for efficient in **vitro sialylation** of saccharide groups attached to glycoproteins, in particular recombinantly produced glycoproteins. For example, the methods of the invention are useful for sialylation of recombinantly produced therapeutic glycoproteins that are incompletely sialylated during production in mammalian cells or transgenic animals. The methods involve contacting the saccharide groups with a sialyltransferase and a sialic acid donor moiety for a sufficient time and under appropriate reaction conditions to transfer sialic acid from the sialic acid donor moiety to the saccharide groups. Sialyltransferases comprise a family of glycosyltransferases that transfer sialic acid from the donor substrate CMP-sialic acid to acceptor oligosaccharide substrates. In preferred embodiments, the sialyltransferases used in the methods of the invention are recombinantly produced.

Detail Description Paragraph - DETX (27):
[0040] The in **vitro sialylation** methods provided by the invention are, unlike previously described sialylation methods, practical for commercial-scale production of modified glycoproteins. Thus, the claimed methods provide a practical means for large-scale preparation of glycoproteins having altered sialylation patterns. The methods are well suited for therapeutic glycoproteins that are incompletely sialylated during production in mammalian cells or transgenic animals. The processes provide an increased and consistent level of terminal sialylation of a glycoprotein.

Detail Description Paragraph - DETX (42):
[0055] Thus, in one embodiment, the invention provides methods for in **vitro sialylation** of saccharide groups present on a glycoprotein that first involves modifying the glycoprotein to create a suitable acceptor. A preferred method for synthesizing an acceptor involves use of a galactosyltransferase. The steps for these methods include:

Claims Text - CLTX (33):

32. A method for in **vitro sialylati n** of saccharide groups present on a glycoprotein, said method comprising contacting said saccharide groups with a sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase activity for a sufficient time and under appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said saccharide group, wherein said sialyltransferase is present at a concentration about 50 mU per mg of glycoprotein or less.

Claims Text - CLTX (58):

57. A method for in **vitro sialylation** of saccharide groups present on a glycoprotein, the method comprising contacting the saccharide groups with an ST3Gal III sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase activity for a sufficient time and under conditions to transfer sialic acid from said sialic acid donor moiety to said saccharide group, wherein said ST3Gal III sialyltransferase is present at a concentration of less than about 50 mU per mg of glycoprotein.

PGPUB-DOCUMENT-NUMBER: 20020115833

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020115833 A1

TITLE: Erythropoietin conjugates

PUBLICATION-DATE: August 22, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Burg, Josef	Weilheim		DE	
Engel, Alfred	Tutzing		DE	
Franze, Reinhard	Penzberg		DE	
Hilger, Bernd	Penzberg		DE	
Schurig, Hartmut Ernst	Muenchen		DE	
Tischer, Wilhelm	Peissenberg		DE	
Wozny, Manfred	Weilheim		DE	

APPL-NO: 10/ 014363

DATE FILED: December 11, 2001

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
EP	00127891.0	2000EP-00127891.0	December 20, 2000

US-CL-CURRENT: 530/395

ABSTRACT:

The present invention refers to conjugates of erythropoietin with poly(ethylene glycol) comprising an erythropoietin glycoprotein having an N-terminal .alpha.-amino group and having the in vivo biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells and selected from the group consisting of human erythropoietin and analogs thereof which have the sequence of human erythropoietin modified by the addition of from 1 to 6 glycosylation sites or a rearrangement of at least one glycosylation site; said glycoprotein being covalently linked to one poly(ethylene glycol) group of the formula
--CO--(CH.sub.2).sub.x --(OCH.sub.2CH.sub.2).sub.m--OR
wherein the--CO of the poly(ethylene glycol) group forms an amide bond with said N-terminal .alpha.-amino group; and wherein R is lower alkyl; x is 2 or 3; and m is from about 450 to about 1350.

---------- KWIC ---------

Detail Description Paragraph - DETX (27):

[0040] Glycosylation of a protein, with one or more oligosaccharide groups, occurs at specific locations along a polypeptide backbone and greatly affects the physical properties of the protein such as protein stability, secretion, subcellular localisation, and biological activity. Glycosylation is usually of two types. O-linked oligosaccharides are attached to serine or threonine residues and N-linked oligosaccharides are attached to asparagine residues. One type of oligosaccharide found on both N-linked and O-linked oligosaccharides is N-acetylneuraminic acid (sialic acid), which is a family of amino sugars containing 9 or more carbon atoms. Sialic acid is usually the terminal residue on both N-linked and O-linked oligosaccharides and, because it bears a negative charge, confers acidic properties to the glycoprotein. Human erythropoietin, having 165 amino acids, contains three N-linked and one O-linked oligosaccharide chains which comprise about 40% of the total molecular weight of the glycoprotein. N-linked glycosylation occurs at asparagine residues located at positions 24, 38, and 83 and O-linked glycosylation occurs at a serine residue located at position 126. The oligosaccharide chains are modified with terminal sialic acid residues. Enzymatic removal of all sialic acid residues from the glycosylated erythropoietin results in loss of in vivo activity but not in **vitro activity because sialylation** of erythropoietin prevents its binding, and subsequent clearance, by hepatic binding protein.

PGPUB-DOCUMENT-NUMBER: 20020076401

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020076401 A1

TITLE: Heat shock protein inducing factor and method for its in-situ generation from an inactive form

PUBLICATION-DATE: June 20, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Volloch, Vladimir	Brookline	MA	US	

APPL-NO: 09/ 727938

DATE FILED: November 30, 2000

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60234406 20000921 US

US-CL-CURRENT: 424/94.1, 514/553

ABSTRACT:

The present invention provides an active heat shock protein inducing factor which is capable of inducing expression of heat shock proteins such as Hsp70 and Hsp27 on-demand in living mammalian cells. The induction and intracellular expression of such heat shock proteins provides major protection for the cells against injurious stresses and can be used therapeutically for treatment of conditions and disorders caused by cell death via apoptosis or necrosis. The heat shock protein-inducing factor is a naturally occurring molecule to be found in the blood serum of living mammals in two different states and activity forms: an inactive form which circulates freely and systemically as a component of the blood serum; and an active molecule which is generated by conversion from the inactive form via modification in-situ. Such a conversion occurs physiologically as a result of stresses or may be effected in-situ ondemand. The invention includes the means and method for converting the inactive form into the active heat shock protein inducing factor state on-demand under both in-vivo and in-vitro circumstances.

PROVISIONAL PATENT APPLICATION

[0001] This invention was first filed as a Provisional Patent Application on Sep. 21, 2000 as U.S. Serial No. 60/234,406.

---------- KWIC ---------

Detail Description Table CWU - DETL (2):

.quadrature.1-6 linkages very slowly. Rate of hydrolysis decreases rapidly with increasing size and complexity of the substrate. Glc.quadrature.1-96X .quadrature.-Glucuronidase Cleaves terminal glucose acid which is .quadrature.-linked to mono-, oligo .quadrature.-D-Glucoronide glucuronoso- or polysaccharides phenol Hydrolase GlcA.quadrature.1-96X EC 3.2.1.31 .quadrature.-Mannosidase Broad aglycone specificity hydrolyzing Man.quadrature.162 man, man .quadrature.166 .quadrature.-D-Mannoside mannohydrolase Man at 100%, and Man.quadrature.163 Man at 7% reaction rate EC 3.2.1.24 Man.quadrature.1-96X Neuraminidase (Sialidase) Cleaves terminal sialic acid residues which are .quadrature.-2,3-, .quadrature.2,6- or Acyineuraminyl hydrolase .quadrature.2,8-linked to Gal, GlcNAc, GalNAc, AcNeu, GlcNeu, EC 3.2.1.18 oligosaccharides, glycolipids or glycoproteins. Relative rate of cleavage is .quadrature.266 > .quadrature.263 > .quadrature.2 determined on bonds in tri- and tetrasaccharides. AcNeu.quadrature.2-96X or GlcNeu.quadrature.2-96X C. Glycosyltransferases, such as: Galactosyltransferase Transfers galactose residues from UDP-galactose to the C.sub.4-- Lactose synthase hydroxyl of N-acetylglucosamine in glycoproteins. Catalyzes the UDPgalactose: D-glucose synthesis of lactose in combination with a-lactalbumin under 4-B-D-galactosyltransferase formation of a .quadrature.164 linkage EC 2.4.1.22 UDPgalactose: N-acetyl-D-Glycosaminyl-glycopeptide 4-.quadrature.-D-galactosyltransferase EC 2.4.1.90 2,6-Sialyttransferase Transfers sialic acid residues to the 6-position of the CMP-N-acetyineuraminate: Gal.quadrature.164GlcNAc unit of glycoconjugates. The preparation is .quadrature.-D-galactodyl-1,4-N-acetyl- used for in**-vitro sialylation** of Gal.quadrature.(1-4)GlcNAc structures in .quadrature.-D-glucosamine a-2-6-N-acetyl- N-glycans via a(2-6) links neuraminyltransferase EC 2.4.99.1

PGPUB-DOCUMENT-NUMBER: 20020041881

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020041881 A1

TITLE: IDENTIFICATION AND CHARACTERIZATION OF NOVEL PNEUMOCOCCAL CHOLINE BINDING PROTEIN, CBPG, AND DIAGNOSTIC AND THERAPEUTIC USES THEREOF

PUBLICATION-DATE: April 11, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
TUOMANEN, ELAINE I.	GERMANTOWN	TN	US	
GOSINK, KHOOSHEH	CORDOVA	TN	US	
MASURE, ROBERT	GERMANTOWN	TN	US	

APPL-NO: 09/ 287070

DATE FILED: April 6, 1999

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

child 09287070 A1 19990406

parent continuation-in-part-of 09196389 19981119 US ABANDONED

US-CL-CURRENT: 424/190.1

ABSTRACT:

The present invention provides isolated polypeptides comprising an amino acid sequence of a choline binding protein CbpG. This invention provides an isolated polypeptide comprising an amino acid sequence of a choline binding polypeptide CbpG or N-terminal CbpG truncate, including analogs, variants, mutants, derivatives and fragments thereof. This invention further provides an isolated immunogenic polypeptide comprising an amino acid sequence of a choline binding protein CbpG. This invention provides an isolated nucleic acid encoding a polypeptide comprising an amino acid sequence of a choline binding protein CbpG. This invention provides pharmaceutical compositions, vaccines, and diagnostic and therapeutic methods of use of the isolated polypeptides and nucleic acids. Assays for compounds which alter or inactivate the polypeptides of the present invention for use in therapy are also provided.

RELATED APPLICATIONS

[0001] The present application is a continuation-in-part of copending

application Ser. No. 09/196,389 filed Nov. 19, 1998, of which the instant application claims the benefit of the filing date pursuant to 35 U.S.C. .sctn.120, and which is incorporated herein by reference in its entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (168):

[0210] We have tested the ability of this mutant to adhere to human Detroit cells, and to carbohydrates in vitro. The in vitro adhesion experiments were done as previously described (Cundell D. R. et al (1995) Infect Immun 63(3):757-761) and the data shown in Table 2 indicate that there is a 40% reduction in adhesion to Detroit human nasopharyngeal cell line by the CbpG-deficient mutant and a 80-90% reduction in adhesion to both lacto-N-neotrose (LNnT) and **sialylactose in vitro**. These data along with the in vivo colonization data suggest that CbpG plays a role in adherence to cells of the nasopharynx and that the binding may occur through sugars on the eukaryotic cells.

PGPUB-DOCUMENT-NUMBER: 20020037841

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020037841 A1

TITLE: Erythropoietin composition

PUBLICATION-DATE: March 28, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Papadimitriou, Apollon	Bichl		DE	

APPL-NO: 09/ 853731

DATE FILED: May 11, 2001

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
EP	00110355.5	2000EP-00110355.5	May 15, 2000

US-CL-CURRENT: 514/8

ABSTRACT:

The present invention relates to a liquid pharmaceutical composition comprising an erythropoietin protein, a multiple charged inorganic anion in a pharmaceutically acceptable buffer suitable to keep the solution pH in the range from about 5.5 to about 7.0, and optionally one or more pharmaceutically acceptable excipients. This composition is especially useful for the prophylaxis and treatment of diseases related to erythropoiesis.

---------- KWIC ---------

Detail Description Paragraph - DETX (13):

[0053] Further, the erythropoietin product of this invention may be a glycoprotein of the above sequences modified by having from 1 to 6 additional sites for glycosylation. Glycosylation of a protein, with one or more oligosaccharide groups, occurs at specific locations along a polypeptide backbone and greatly affects the physical properties of the protein such as protein stability, secretion, subcellular localization, and biological activity. Glycosylation is usually of two types. O-linked oligosaccharides are attached to serine or threonine residues and N-linked oligosaccharides are attached to asparagine residues. One type of oligosaccharide found on both N-linked and O-linked oligosaccharides is N-acetylneuraminic acid (sialic acid), which is a family of amino sugars containing 9 or more carbon atoms. Sialic acid is usually the terminal residue on both N-linked and O-linked oligosaccharides and, because it bears a negative charge, confers acidic

properties to the glycoprotein. Human erythropoietin, having 165 amino acids, contains three N-linked and one O-linked oligosaccharide chains which comprise about 40% of the total molecular weight of the glycoprotein. N-linked glycosylation occurs at asparagine residues located at positions 24, 38, and 83 and O-linked glycosylation occurs at a serine residue located at position 126. The oligosaccharide chains are modified with terminal sialic acid residues. Enzymatic removal of all sialic acid residues from the glycosylated erythropoietin results in loss of in vivo activity but not in **vitro activity because sialylation** of erythropoietin prevents its binding, and subsequent clearance, by hepatic binding protein.

PGPUB-DOCUMENT-NUMBER: 20020019342

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020019342 A1

TITLE: In vitro modification of glycosylation patterns of recombinant glycopeptides

PUBLICATION-DATE: February 14, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bayer, Robert	San Diego	CA	US	

APPL-NO: 09/ 855320

DATE FILED: May 14, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60203851 20000512 US

US-CL-CURRENT: 514/8, 435/14

ABSTRACT:

This invention provides methods for modifying glycosylation patterns of glycopeptides, including recombinantly produced glycopeptides. Also provided are glycopeptide compositions in which the glycopeptides have a uniform glycosylation pattern.

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of prior U.S. provisional application number 60/203,851, filed May 12, 2000.

---------- KWIC ---------

Detail Description Paragraph - DETX (73):
[0121] Thus, in some embodiments, the invention provides methods for in **vitro sialylation** of saccharide groups present on a glycopeptide that first involves modifying the glycopeptide to create a suitable acceptor. Examples of preferred methods of multi-enzyme synthesis of desired oligosaccharide determinants are as follows.

Detail Description Paragraph - DETX (128):
[0175] The present examples exemplify the methods of the invention. Example

1 sets forth the introduction of sialyl Lewis x structures onto a peptide using **sialylation and fucosylation in vitro**. Example 2 sets forth the results of an investigation into the substrated specificity and fucosylation activity of two fucosyltransferases, FucT-V and FucT-VI. Example 3 sets forth an exemplary fucosylation process of the invention utilizing the protein RsCD4 as a substrate for fucosylation. The fucosylation step is preceded by a sialylation step. Example 4 sets forth an exemplar assay for determining the ability of a fucosyltransferase to act on a particular glycoprotein.

Detail Description Paragraph - DETX (130):

[0176] Example 1 sets forth the introduction of sialyl Lewis x structures onto a peptide using using **sialylation and fucosylation in vitro**.

US-PAT-NO: 6583272

DOCUMENT-IDENTIFIER: US 6583272 B1

TITLE: Erythropoietin conjugates

DATE-ISSUED: June 24, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bailon; Pascal Sebastian	Florham Park	NJ	N/A	N/A

APPL-NO: 09/ 604938

DATE FILED: June 27, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

The priority of U.S. Provisional Application No. 60/142,254, filed Jul. 2, 1999; No. 60/150,225, filed Aug. 23, 1999; No. 60/151,548, filed Aug. 31, 1999; and No. 60/166,151, filed Nov. 17, 1999 is claimed.

US-CL-CURRENT: 530/397, 424/194.1 , 424/195.11 , 530/350

ABSTRACT:

Conjugates of erythropoietin with poly(ethylene glycol) comprise an erythropoietin glycoprotein having at least one free amino group and having the in vivo biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells and selected from the group consisting of human erythropoietin and analogs thereof which have sequence of human erythropoietin modified by the addition of from 1 to 6 glycosylation sites or a rearrangement of at least one glycosylation site; the glycoprotein being covalently linked to "n" poly(ethylene glycol) groups of the formula --CO--(CH.sub.2).sub.x (OCH.sub.2 CH.sub.2).sub.m --OR with the carbonyl of each poly(ethylene glycol) group forming an amide bond with one of said amino groups; wherein R is lower alkyl; x is 2 or 3; m is about 450 to about 900; n is from 1 to 3; and n and m are chosen so that the molecular weight of the conjugate minus the erythropoietin glycoprotein is from 20 kilodaltons to 100 kilodaltons.

15 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

---------- KWIC ---------

Detailed Description Text - DETX (8):

In an embodiment, P may be the residue of a glycoprotein analog having from 1 to 6 additional sites for glycosylation. Glycosylation of a protein, with one or more oligosaccharide groups, occurs at specific locations along a polypeptide backbone and greatly affects the physical properties of the protein such as protein stability, secretion, subcellular localization, and biological activity. Glycosylation is usually of two types. O-linked oligosaccharides are attached to serine or threonine residues and N-linked oligosaccharides are attached to asparagine residues. One type of oligosaccharide found on both N-linked and O-linked oligosaccharides is N-acetylneuraminic acid (sialic acid), which is a family of amino sugars containing 9 or more carbon atoms. Sialic acid is usually the terminal residue on both N-linked and O-linked oligosaccharides and, because it bears a negative charge, confers acidic properties to the glycoprotein. Human erythropoietin, having 165 amino acids, contains three N-linked and one O-linked oligosaccharide chains which comprise about 40% of the total molecular weight of the glycoprotein. N-linked glycosylation occurs at asparagine residues located at positions 24, 38, and 83 and O-linked glycosylation occurs at a serine residue located at position 126. The oligosaccharide chains are modified with terminal sialic acid residues. Enzymatic removal of all sialic acid residues from the glycosylated erythropoietin results in loss of in vivo activity but not in **vitro activity because sialylation** of erythropoietin prevents its binding, and subsequent clearance, by hepatic binding protein.

US-PAT-NO: 6495139

DOCUMENT-IDENTIFIER: US 6495139 B2
See image for Certificate of Correction

TITLE: Identification and characterization of novel pneumococcal choline binding protein, CBPG, and diagnostic and therapeutic uses thereof

DATE-ISSUED: December 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tuomanen; Elaine I.	Germantown	TN	N/A	N/A
Gosink; Khoosheh	Cordova	TN	N/A	N/A
Masure; Robert	Germantown	TN	N/A	N/A

APPL-NO: 09/ 287070

DATE FILED: April 6, 1999

PARENT-CASE:

RELATED APPLICATIONS

The present application is a continuation-in-part of application Ser. No. 09/196,389 filed Nov. 19, 1998, now abandoned, of which the instant application claims the benefit of the filing date pursuant to 35 U.S.C. .sctn.120, and which is incorporated herein by reference in its entirety.

US-CL-CURRENT: 424/190.1, 424/184.1 , 424/185.1 , 424/244.1 , 530/300 , 530/324 , 530/350

ABSTRACT:

The present invention provides isolated polypeptides comprising an amino acid sequence of a choline binding protein CbpG. This invention provides an isolated polypeptide comprising an amino acid sequence of a choline binding polypeptide CbpG or N-terminal CbpG truncate, including analogs, variants, mutants, derivatives and fragments thereof This invention further provides an isolated immunogenic polypeptide comprising an amino acid sequence of a choline binding protein CbpG. This invention provides an isolated nucleic acid encoding a polypeptide comprising an amino acid sequence of a choline binding protein CbpG. This invention provides pharmaceutical compositions, vaccines, and diagnostic and therapeutic methods of use of the isolated polypeptides and nucleic acids. Assays for compounds which alter or inactivate the polypeptides of the present invention for use in therapy are also provided.

6 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

---------- KWIC ---------

Detailed Description Text - DETX (159):

We have tested the ability of this mutant to adhere to human Detroit cells, and to carbohydrates in vitro. The in vitro adhesion experiments were done as previously described (Cundell D. R. et al (1995) Infect Immun 63(3):757-761) and the data shown in Table 2 indicate that there is a 40% reduction in adhesion to Detroit human nasopharyngeal cell line by the CbpG-deficient mutant and a 80-90% reduction in adhesion to both lacto-N-neotrose (LNnT) and **sialylactose in vitro**. These data along with the in vivo colonization data suggest that CbpG plays a role in adherence to cells of he nasopharynx and that the binding may occur through sugars on the eukaryotic cells.

US-PAT-NO: 6440703

DOCUMENT-IDENTIFIER: US 6440703 B1
See image for Certificate of Correction

TITLE: Enzymatic synthesis of gangliosides

DATE-ISSUED: August 27, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
DeFrees; Shawn	San Marcos	CA	N/A	N/A

APPL-NO: 09/ 935363

DATE FILED: August 22, 2001

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 09/203,200 filed on Nov. 30, 1998 now abandoned and it claims the benefit of U.S. Provisional Application No. 60/067,693, filed Dec. 1, 1997, the disclosures of both of which are incorporated herein by reference in their entirety for all purposes.

US-CL-CURRENT: 435/84, 435/72 , 435/74 , 435/97

ABSTRACT:

This invention provides methods for practical in vitro synthesis of gangliosides and other glycolipids. The synthetic methods typically involve enzymatic synthesis, or a combination of enzymatic and chemical synthesis. One or more of the enzymatic steps is preferably carried out in the presence of an organic solvent.

18 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

---------- KWIC ---------

Detailed Description Text - DETX (34):
In some embodiments, the invention provides methods for in **vitro sialylation** of saccharide groups present on a glycosylceramide, wherein the methods first

involve modifying the glycosylceramide to create a suitable acceptor. A preferred method for synthesizing an acceptor involves use of a galactosyltransferase. The steps for these methods include:

US-PAT-NO: 6399336

DOCUMENT-IDENTIFIER: US 6399336 B1

TITLE: Practical in **vitro sialylation** of recombinant glycoproteins

DATE-ISSUED: June 4, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Paulson; James C.	Del Mar	CA	N/A	N/A
Bayer; Robert J.	San Diego	CA	N/A	N/A
Sjoberg; Eric	San Diego	CA	N/A	N/A

APPL-NO: 09/ 007741

DATE FILED: January 15, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 60/035,710, filed Jan. 16, 1997, which is incorporated herein by reference in its entirety for all purposes.

US-CL-CURRENT: 435/97, 435/15 , 435/183 , 435/193 , 435/220 , 435/252.3 , 435/4 , 435/41 , 435/7.2

ABSTRACT:

This invention provides methods for practical in **vitro sialylation** of glycoproteins, including recombinantly produced glycoproteins. The methods are useful for large-scale modification of sialylation patterns.

87 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

---------- KWIC ---------

Abstract Text - ABTX (1):

This invention provides methods for practical in **vitro sialylation** of glycoproteins, including recombinantly produced glycoproteins. The methods are useful for large-scale modification of sialylation patterns.

TITLE - TI (1):
Practical in **vitro sialylation** of recombinant glycoproteins

Brief Summary Text - BSTX (3):
This invention pertains to the field of in **vitro sialylation** of glycoproteins, including recombinant glycoproteins.

Brief Summary Text - BSTX (10):
The present invention provides methods for in **vitro sialylation** of saccharide groups present on a recombinantly produced glycoprotein. The methods comprise contacting the saccharide groups with a sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase activity for a sufficient time and under appropriate conditions to transfer sialic acid from the sialic acid donor moiety to said saccharide group.

Detailed Description Text - DETX (23):
The present invention provides methods for efficient in **vitro sialylation** of saccharide groups attached to glycoproteins, in particular recombinantly produced glycoproteins. For example, the methods of the invention are useful for sialylation of recombinantly produced therapeutic glycoproteins that are incompletely sialylated during production in mammalian cells or transgenic animals. The methods involve contacting the saccharide groups with a sialyltransferase and a sialic acid donor moiety for a sufficient time and under appropriate reaction conditions to transfer sialic acid from the sialic acid donor moiety to the saccharide groups. Sialyltransferases comprise a family of glycosyltransferases that transfer sialic acid from the donor substrate CMP-sialic acid to acceptor oligosaccharide substrates. In preferred embodiments, the sialyltransferases used in the methods of the invention are recombinantly produced.

Detailed Description Text - DETX (27):
The in **vitro sialylation** methods provided by the invention are, unlike previously described sialylation methods, practical for commercial-scale production of modified glycoproteins. Thus, the claimed methods provide a practical means for large-scale preparation of glycoproteins having altered sialylation patterns. The methods are well suited for therapeutic glycoproteins that are incompletely sialylated during production in mammalian cells or transgenic animals. The processes provide an increased and consistent level of terminal sialylation of a glycoprotein.

Detailed Description Text - DETX (42):
Thus, in one embodiment, the invention provides methods for in **vitro sialylation** of saccharide groups present on a glycoprotein that first involves modifying the glycoprotein to create a suitable acceptor. A preferred method for synthesizing an acceptor involves use of a galactosyltransferase. The steps for these methods include:

Claims Text - CLTX (32):

32. A large-scale method for in **vitro sialylation** of saccharide group s present on a glycoprotein, said method comprising contacting said saccharide groups with a sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase activity for a sufficient time and under appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said saccharide group, wherein said sialyltransferase is present at a concentration about 50 mU per mg of glycoprotein or less, and wherein at least about 80% of the saccharide groups are sialylated.

Claims Text - CLTX (57):

57. A large-scale method for in **vitro sialylation** of terminal galactose residues present on a glycoprotein, said method comprising contacting said glycoprotein with a reaction mixture that comprises a sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase activity, for a sufficient time and under appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said terminal galactose residues, wherein at least about 80% of the terminal galactose residues present on the glycoprotein are sialylated, and wherein said sialyltransferase is present at a concentration about 50 mU per mg of glycoprotein or less.

Claims Text - CLTX (59):

59. A large-scale method for in **vitro sialylation** of terminal galactose residues present on a glycoprotein, said method comprising contacting said glycoprotein with a reaction mixture that comprises a sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase activity, for a sufficient time and under appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said terminal galactose residues, wherein a greater percentage of terminal galactose residues are sialylated compared to an unaltered glycoprotein, wherein said greater percentage is equal to at least about 80%, and wherein said sialyltransferase is present at a concentration about 50 mU per mg of glycoprotein or less.

US-PAT-NO: 5908754

DOCUMENT-IDENTIFIER: US 5908754 A

TITLE: Method for in vitro determination of in vivo erythropoeitin bioactivity

DATE-ISSUED: June 1, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lisi; Peter J.	Flemington	NJ	N/A	N/A
Glenn; Jeffrey K.	Middlesex	NJ	N/A	N/A
So; Chi-Kwong	Somerville	NJ	N/A	N/A

APPL-NO: 08/ 948387

DATE FILED: October 10, 1997

PARENT-CASE:

This application is a continuation of application Ser. No. 08/772,995 filed Dec. 24, 1996, now abandoned, which is a continuation of application Ser. No. 08/495,730, filed Jun. 8, 1995, now abandoned; which is a continuation of Ser. No. 08/107,390, filed Aug. 16, 1993, now abandoned.

US-CL-CURRENT: 435/6, 435/4 , 435/7.1

ABSTRACT:

The present invention is directed to an in vitro method of determining the in vivo EPO activity of a sample containing EPO. More particularly, the present method comprises treating a sample containing EPO under conditions which remove desialylated EPO, and measuring the in vitro EPO activity of the resulting treated sample. In a preferred embodiment, desialylated EPO is removed from the sample by incubating the sample with cells of the human hepatoma cell line HepG2, and in vitro EPO activity is determined by incubating the treated sample with cells of an EPO-responsive cell line and measuring the proliferation or viability of the EPO-responsive cells. The present invention is useful, for example, in quantitating the biologically active EPO in a variety of sample types.

12 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (17):

In vitro EPO activity is defined as the ability to stimulate the proliferation or viability, in vitro, of cells which express a functional EPO-receptor, for example, erythroid precursor cells. As discussed hereinbelow, both **sialylated and desialylated EPO are active in vitro**. In vivo EPO activity is defined as the ability to stimulate the proliferation of erythroid precursor cells in vivo. Desialylated EPO is considered herein to be essentially inactive in vivo due to rapid hepatic clearance.

US-PAT-NO: 5096816

DOCUMENT-IDENTIFIER: US 5096816 A

TITLE: In vitro management of ammonia's effect on glycosylation of cell products through pH control

DATE-ISSUED: March 17, 1992

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Maiorella; Brian L.	Oakland	CA	N/A	N/A

APPL-NO: 07/ 533688

DATE FILED: June 5, 1990

US-CL-CURRENT: 435/70.21

ABSTRACT:

This invention relates generally to the field of cell culture. The invention presents pH based methods for controlling the glycosylation patterns of cell products, particularly proteins produced by cells in vitro, preferably via a multi-level pH control strategy. Preferably, this invention is applied to manage the effect of high level of ammonia on the glycosylation of cell products. The glycosylation affected is preferably that of terminal sialylations of the oligosaccharide of glycoproteins.

2 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Detailed Description Text - DETX (25):

It would be apparent to those skilled in the art that the converse is true. For example, these methods also allow the production of a cell product with decreased terminal **sialylation by providing an in vitro** condition of high level of ammonia at the appropriate pH. Further, it would be apparent to those skilled in the art that the invention can be practiced in any culture condition that produces or has high level of ammonia.

	Hits	Search Text	DBs	Time Stamp
1	21	vitro near4 sialyla$	USPAT; US-PGPUB	2003/12/05 14:52
2	27	(commercial or scale or batch) near4 sialyl$	USPAT; US-PGPUB	2003/12/05 14:53

PGPUB-DOCUMENT-NUMBER: 20030186414

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: **US 20030186414 A1**

TITLE: Nucleic acid that encodes a fusion protein

PUBLICATION-DATE: October 2, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Gilbert, Michel	Hull	CA		
Young, N. Martin	Gloucester		CA	
Wakarchuk, Warren W.	Gloucester		CA	

APPL-NO: 10/ 317428

DATE FILED: December 11, 2002

RELATED-US-APPL-DATA:

child 10317428 A1 20021211

parent division-of 09211691 19981214 US PENDING

non-provisional-of-provisional 60069443 19971215 US

US-CL-CURRENT: 435/193, 435/320.1 , 435/325 , 435/6 , 435/69.1 , 536/23.2

ABSTRACT:

This invention provides fusion polypeptides that include a glycosyltransferase catalytic domain and a catalytic domain from an accessory enzyme that is involved in making a substrate for a glycosyltransferase reaction. Nucleic acids that encode the fusion polypeptides are also provided, as are host cells for expressing the fusion polypeptides of the invention.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of, and claims benefit of, U.S. Provisional Application No. 60/069,443, filed Dec. 15, 1997, which application is incorporated herein by reference for all purposes.

---------- KWIC ---------

Pre-Grant Publication Document Identifier - DID (1):
US 20030186414 A1

Detail Description Paragraph - DETX (128):

[0138] This Example describes the construction and expression of a polynucleotide that encodes a fusion protein that has both CMP-Neu5Ac synthetase activity and .alpha.2,3-sialyltransferase activity. Large-scale enzymatic synthesis of oligosaccharides containing terminal N-acetyl-neuraminic acid residues requires large amounts of the sialyltransferase and the corresponding sugar-nucleotide synthetase for the synthesis of the sugar-nucleotide donor, CMP-Neu5Ac, an unstable compound. Using genes cloned from Neisseria meningitidis, we constructed a fusion protein which has both CMP-Neu5Ac synthetase and .alpha.-2,3-sialyltransferase activities. The fusion protein was produced in high yields (over 1,200 units per liter, measured using an .alpha.-2,3-sialyltransferase assay) in Escherichia coli and functionally pure enzyme could be obtained using a simple protocol. In small-scale enzymatic syntheses, we showed that the fusion protein could sialylate various oligosaccharide acceptors (branched and linear) with N-acetyl-neuraminic acid as well as N-glycolyl- and N-propionyl-neuraminic acid in high conversion yield. The fusion protein was also used to produce .alpha.-2,3-**sialyllactose at the 100 g scale** using a sugar nucleotide cycle reaction, starting from lactose, sialic acid, phosphoenolpyruvate and catalytic amounts of ATP and CMP.

Detail Description Paragraph - DETX (151):

[0161] E. coli BMH71-18 was transformed with the three versions of pFUS-01 and the level of .alpha.-2,3-**sialyltransferase activity was compared in small-scale** cultures (20 mL). The highest activity was obtained with pFUS-01/2, which gave 40% more activity than pFUS-01/4 and 60% more activity than pFUS-01. The fusion protein encoded by pFUS-01/2 has the longest linker which might aid the independent folding of the two components. However, the effects of linker composition and length were not further studied and pFUS-01/2 was used for the scale-up in production and kinetics comparison.

Detail Description Paragraph - DETX (167):

[0177] To be useful for large scale carbohydrate synthesis the fusion protein should be applicable in a sugar nucleotide cycle. This cycle is designed to use only catalytic amounts of expensive sugar nucleotides and nucleoside phosphates, which are enzymatically regenerated in situ from low-cost precursors. The recycling of the converted co-factors also prevents end-product inhibition. The .alpha.-2,3-**sialyllactose 100 g scale** synthesis went to completion, which is important since stoichiometric conversion of substrates is desirable not only to minimize reagent costs but also because it greatly simplifies the purification of the product from a large scale synthesis. Another interesting feature of the fusion protein is that it can use directly different donor analogs and various acceptors with a terminal galactose residue. Consequently it can be used for the synthesis of both natural carbohydrates and synthetic derivatives with novel properties.

PGPUB-DOCUMENT-NUMBER: 20030180928

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: **US 20030180928 A1**

TITLE: Fusion protein comprising a UDP-Galnac 4' epimerase and a galnac transferase

PUBLICATION-DATE: September 25, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Gilbert, Michel	Hull	CA		
Young, N. Martin	Gloucester	CA		
Wakarchuk, Warren W.	Gloucester	CA		

APPL-NO: 10/ 317773

DATE FILED: December 11, 2002

RELATED-US-APPL-DATA:

child 10317773 A1 20021211

parent division-of 09211691 19981214 US PENDING

non-provisional-of-provisional 60069443 19971215 US

US-CL-CURRENT: 435/193, 435/320.1 , 435/325 , 435/6 , 435/69.7 , 536/23.2

ABSTRACT:

This invention provides fusion polypeptides that include a glycosyltransferase catalytic domain and a catalytic domain from an accessory enzyme that is involved in making a substrate for a glycosyltransferase reaction. Nucleic acids that encode the fusion polypeptides are also provided, as are host cells for expressing the fusion polypeptides of the invention.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of, and claims benefit of, U.S. Provisional Application No. 60/069,443, filed Dec. 15, 1997, which application is incorporated herein by reference for all purposes.

---------- KWIC ---------

Pre-Grant Publication Document Identifier - DID (1):
US 20030180928 A1

Detail Description Paragraph - DETX (128):

[0138] This Example describes the construction and expression of a polynucleotide that encodes a fusion protein that has both CMP-Neu5Ac synthetase activity and .alpha.2,3-sialyltransferase activity. Large-scale enzymatic synthesis of oligosaccharides containing terminal N-acetyl-neuraminic acid residues requires large amounts of the sialyltransferase and the corresponding sugar-nucleotide synthetase for the synthesis of the sugar-nucleotide donor, CMP-Neu5Ac, an unstable compound. Using genes cloned from Neisseria meningitidis, we constructed a fusion protein which has both CMP-Neu5Ac synthetase and .alpha.-2,3-sialyltransferase activities. The fusion protein was produced in high yields (over 1,200 units per liter, measured using an .alpha.-2,3-sialyltransferase assay) in Escherichia coli and functionally pure enzyme could be obtained using a simple protocol. In small-scale enzymatic syntheses, we showed that the fusion protein could sialylate various oligosaccharide acceptors (branched and linear) with N-acetyl-neuraminic acid as well as N-glycolyl- and N-propionyl-neuraminic acid in high conversion yield. The fusion protein was also used to produce .alpha.-2,3-**sialyllactose at the 100 g scale** using a sugar nucleotide cycle reaction, starting from lactose, sialic acid, phosphoenolpyruvate and catalytic amounts of ATP and CMP.

Detail Description Paragraph - DETX (151):

[0161] E. coli BMH71-18 was transformed with the three versions of pFUS-01 and the level of .alpha.-2,3-**sialyltransferase activity was compared in small-scale** cultures (20 mL). The highest activity was obtained with pFUS-01/2, which gave 40% more activity than pFUS-01/4 and 60% more activity than pFUS-01. The fusion protein encoded by pFUS-01/2 has the longest linker which might aid the independent folding of the two components. However, the effects of linker composition and length were not further studied and pFUS-01/2 was used for the scale-up in production and kinetics comparison.

Detail Description Paragraph - DETX (167):

[0177] To be useful for large scale carbohydrate synthesis the fusion protein should be applicable in a sugar nucleotide cycle. This cycle is designed to use only catalytic amounts of expensive sugar nucleotides and nucleoside phosphates, which are enzymatically regenerated in situ from low-cost precursors. The recycling of the converted co-factors also prevents end-product inhibition. The .alpha.-2,3-**sialyllactose 100 g scale** synthesis went to completion, which is important since stoichiometric conversion of substrates is desirable not only to minimize reagent costs but also because it greatly simplifies the purification of the product from a large scale synthesis. Another interesting feature of the fusion protein is that it can use directly different donor analogs and various acceptors with a terminal galactose residue. Consequently it can be used for the synthesis of both natural carbohydrates and synthetic derivatives with novel properties.

PGPUB-DOCUMENT-NUMBER: 20030180835

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: **US 20030180835 A1**

TITLE: In vitro modification of glycosylation patterns of recombinant glycopeptides

PUBLICATION-DATE: September 25, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bayer, Robert J.	San Diego	CA	US	

APPL-NO: 10/ 391035

DATE FILED: March 17, 2003

RELATED-US-APPL-DATA:

child 10391035 A1 20030317

parent continuation-of 09855320 20010514 US PENDING

non-provisional-of-provisional 60203851 20000512 US

US-CL-CURRENT: 435/68.1, 435/193 , 530/395

ABSTRACT:

This invention provides methods for modifying glycosylation patterns of glycopeptides, including recombinantly produced glycopeptides. Also provided are glycopeptide compositions in which the glycopeptides have a uniform glycosylation pattern.

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Pre-Grant Publication Document Identifier - DID (1):
US 20030180835 A1

Detail Description Paragraph - DETX (90):
[0138] In some embodiments, the invention **sialylation methods that have increased commercial practicality through the use of bacterial sialyltransferases,** either recombinantly produced or produced in the native bacterial cells. Two bacterial sialyltransferases have been recently reported; an ST6Gal II from Photobacterium damsela (Yamamoto et al. (1996) J. Biochem. 120: 104-110) and an ST3Gal V from Neisseria meningitidis (Gilbert et al.

(1996) J. Biol. Chem. 271: 28271-28276). The two recently described bacterial enzymes transfer sialic acid to the Gal.beta.1,4GlcNAc sequence on oligosaccharide substrates. Table 4 shows the acceptor specificity of these and other sialyltransferases useful in the methods of the invention.

Detail Description Paragraph - DETX (93):

[0141] Other sialyltransferases, including those listed above, are also useful in an economic and efficient large **scale process for sialylation** of commercially important glycopeptides. As described above, a simple test to find out the utility of these other enzymes, is to react various amounts of each enzyme (1-100 mU/mg protein) with a readily available glycopeptide protein such as asialo-.alpha..sub.1-AGP (at 1-10 mg/ml) to compare the ability of the sialyltransferase of interest to sialylate glycopeptides. The results can be compared to, for example, either or both of an ST6Gal I or an ST3Gal III (e.g., a bovine or human enzyme), depending upon the particular sialic acid linkage that is desired. Alternatively, other glycopeptides or glycopeptides, or N- or O-linked oligosaccharides enzymatically released from the peptide backbone can be used in place of asialo-.alpha..sub.1 AGP for this evaluation, or one can use saccharides that are produced by other methods or purified from natural products such as milk. Preferably, however, the sialyltransferases are assayed using an oligosaccharide that is linked to a glycopeptide. Sialyltransferases showing an ability to, for example, sialylate N-linked or O-linked oligosaccharides of glycopeptides more efficiently than ST6Gal I are useful in a practical large **scale process for glycopeptide sialylation**.

PGPUB-DOCUMENT-NUMBER: 20030143567

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: **US 20030143567 A1**

TITLE: Methods for enzymatic conversion of GDP-mannose to GDP-fucose

PUBLICATION-DATE: July 31, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sjoberg, Eric R.	San Diego	CA	US	

APPL-NO: 10/ 206655

DATE FILED: July 25, 2002

RELATED-US-APPL-DATA:

child 10206655 A1 20020725

parent division-of 09231905 19990114 US GRANTED

parent-patent 6500661 US

non-provisional-of-provisional 60071076 19980115 US

US-CL-CURRENT: 435/6, 435/189 , 435/252.33 , 435/320.1 , 435/69.1 , 435/91.1 , 536/23.2

ABSTRACT:

This invention provides methods for practical enzymatic conversion of GDP-mannose to GDP-fucose. These methods are useful for efficient synthesis of reactants used in the synthesis of fucosylated oligosaccharides.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Provisional Application No. 60/071,076, filed Jan. 15, 1998, which application is incorporated herein by reference for all purposes.

---------- KWIC ---------

Pre-Grant Publication Document Identifier - DID (1):
US 20030143567 A1

Brief Description of Drawings Paragraph - DRTX (13):

[0038] FIG. 12 shows a time course of a large-**scale sialyl** Lewis X synthesis reaction. Samples were taken from the reaction mixture and analyzed by TLC. From left to right, the lanes are as follows: Lane 1, reaction at 160 hours; Lane 2, 147 hours; Lane 3, SLN; Lane 4, 43 hours; Lane 5, 1 hour.

Detail Description Paragraph - DETX (207):

[0242] To demonstrate the utility of bacterial GDP-mannose dehydratase and YEF B for the large **scale synthesis of sialyl** Lewis X antigen and other bioactive carbohydrates, a 100 gram reaction was conducted in which Neu5Ac.alpha.2,3Gal.beta.1,4GlcNAc.beta.1,3Gal-OR (SLN), the tetrasaccharide precursor of sialyl Lewis X antigen, was fucosylated to form sialyl Lewis X antigen. Conversion of SLN into sialyl Lewis X antigen was monitored by HPLC, TLC and pH by pH electrode. The results of this assay are shown in FIG. 10, in which formation of sialyl Lewis X is monitored by detecting the increase in free GDP concentration. The pH of the reaction mixture is also shown. As GDP was hydrolyzed by alkaline phosphatase liberating inorganic phosphate, the pH of the reaction decreased. The liberated inorganic phosphate also formed a precipitate with magnesium ions, decreasing the concentration of free magnesium, as shown in FIG. 11.

PGPUB-DOCUMENT-NUMBER: 20030134403

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: **US 20030134403 A1**

TITLE: Nucleic acids useful for enzymatic conversion of GDP-mannose to GDP-fucose

PUBLICATION-DATE: July 17, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sjoberg, Eric R.	San Diego	CA	US	

APPL-NO: 10/ 206485

DATE FILED: July 25, 2002

RELATED-US-APPL-DATA:

child 10206485 A1 20020725

parent division-of 09231905 19990114 US GRANTED

parent-patent 6500661 US

non-provisional-of-provisional 60071076 19980115 US

US-CL-CURRENT: 435/189, 435/252.3 , 435/320.1 , 435/69.1 , 435/89 , 536/23.2

ABSTRACT:

This invention provides methods for practical enzymatic conversion of GDP-mannose to GDP-fucose. These methods are useful for efficient synthesis of reactants used in the synthesis of fucosylated oligosaccharides.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Provisional Application No. 60/071,076, filed Jan. 15, 1998, which application is incorporated herein by reference for all purposes.

---------- KWIC ---------

Pre-Grant Publication Document Identifier - DID (1):
US 20030134403 A1

Brief Description of Drawings Paragraph - DRTX (13):

[0038] FIG. 12 shows a time course of a large-**scale sialyl** Lewis X synthesis reaction. Samples were taken from the reaction mixture and analyzed by TLC. From left to right, the lanes are as follows: Lane 1, reaction at 160 hours; Lane 2, 147 hours; Lane 3, SLN; Lane 4, 43 hours; Lane 5, 1 hour.

Detail Description Paragraph - DETX (208):

[0240] To demonstrate the utility of bacterial GDP-mannose dehydratase and YEF B for the large **scale synthesis of sialyl** Lewis X antigen and other bioactive carbohydrates, a 100 gram reaction was conducted in which Neu5Ac.alpha.2,3Gal.beta.1,4GlcNAc.beta.1,3Gal-OR (SLN), the tetrasaccharide precursor of sialyl Lewis X antigen, was fucosylated to form sialyl Lewis X antigen. Conversion of SLN into sialyl Lewis X antigen was monitored by HPLC, TLC and pH by pH electrode. The results of this assay are shown in FIG. 10, in which formation of sialyl Lewis X is monitored by detecting the increase in free GDP concentration. The pH of the reaction mixture is also shown. As GDP was hydrolyzed by alkaline phosphatase liberating inorganic phosphate, the pH of the reaction decreased. The liberated inorganic phosphate also formed a precipitate with magnesium ions, decreasing the concentration of free magnesium, as shown in FIG. 11.

PGPUB-DOCUMENT-NUMBER: 20030124645

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: **US 20030124645 A1**

TITLE: Practical in vitro sialylation of recombinant glycoproteins

PUBLICATION-DATE: July 3, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Paulson, James C.	Del Mar	CA	US	
Bayer, Robert J.	San Diego	CA	US	
Sjoberg, Eric	San Diego	CA	US	

APPL-NO: 10/ 219120

DATE FILED: August 13, 2002

RELATED-US-APPL-DATA:

child 10219120 A1 20020813

parent continuation-of 10007331 20011109 US PENDING

child 10007331 20011109 US

parent division-of 09007741 19980115 US GRANTED

parent-patent 6399336 US

non-provisional-of-provisional 60035710 19970116 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
WO	PCT/US98/00835	1998WO-PCT/US98/00835	January 15, 1998

US-CL-CURRENT: 435/68.1, 536/53

ABSTRACT:

This invention provides methods for practical in vitro sialylation of glycoproteins, including recombinantly produced glycoproteins. The methods are useful for large-**scale modification of sialylation** patterns.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 60/035,710, filed Jan. 16, 1997, which is incorporated herein by reference in

its entirety for all purposes.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):
This invention provides methods for practical in vitro sialylation of glycoproteins, including recombinantly produced glycoproteins. The methods are useful for large-**scale modification of sialylation** patterns.

Pre-Grant Publication Document Identifier - DID (1):
US 20030124645 A1

Detail Description Paragraph - DETX (18):
[0031] The in vitro sialylation methods provided by the invention are, unlike previously described **sialylation methods, practical for commercial**-scale production of modified glycoproteins. Thus, the claimed methods provide a practical means for large-**scale preparation of glycoproteins having altered sialylation** patterns. The methods are well suited for therapeutic glycoproteins that are incompletely sialylated during production in mammalian cells or transgenic animals. The processes provide an increased and consistent level of terminal sialylation of a glycoprotein.

Detail Description Paragraph - DETX (26):
[0039] Other sialyltransferases, including those listed in Table 1, may also be useful in an economic and efficient large **scale process for sialylation** of commercially important glycoproteins. As a simple test to find out the utility of these other enzymes, various amounts of each enzyme (1-100 mU/mg protein) are reacted with asialo-.alpha..sub.1 AGP (at 1-10 mg/ml) to compare the ability of the sialyltransferase of interest to sialylate glycoproteins relative to either bovine ST6Gal I, ST3Gal III or both sialyltransferases. Alternatively, other glycoproteins or glycopeptides, or N-linked oligosaccharides enzymatically released from the peptide backbone can be used in place of asialo-.alpha..sub.1 AGP for this evaluation. Sialyltransferases showing an ability to sialylate N-linked oligosaccharides of glycoproteins more efficiently than ST6Gal I may prove useful in a practical large **scale process for glycoprotein sialylation** (as illustrated for ST3Gal III in this disclosure).

Detail Description Paragraph - DETX (59):
[0070] This Example demonstrates that sialylation of recombinant glycoproteins using the ST3Gal III sialyltransferase requires much less enzyme than expected. For a one kilogram scale reaction, approximately 7,000 units of the ST3Gal III sialyltransferase would be needed instead of 100,000-150,000 units that the earlier studies indicated. Purification of these enzymes from natural sources is prohibitive, with yields of only 1-10 units for a large scale preparation after 1-2 months work. Assuming that both the ST6Gal I and ST3Gal III sialyltransferases are produced as recombinant sialyltransferases,

with equal levels of expression of the two enzymes being achieved, a fermentation scale 14-21 times greater (or more) would be required for the ST6Gal I sialyltransferase relative to the ST3Gal III sialyltransferase. For the ST6Gal I sialyltransferase, expression levels of 0.3 U/1 in yeast has been reported. Borsig et al. (1995) Biochem. Biophys. Res. Commun. 210: 14-20. Expression levels of 1000 U/liter of the ST3Gal III sialyltransferase have been achieved in Aspergillus niger. At current levels of expression 300-450,000 liters of yeast fermentation would be required to produce sufficient enzyme for sialylation of 1 kg of glycoprotein using the ST6Gal I sialyltransferase. In contrast, less than 10 liter fermentation of Aspergillus niger would be required for sialylation of 1 kg of glycoprotein using the ST3Gal III sialyltransferase. Thus, the fermentation capacity required to produce the ST3Gal III **sialyltransferase for a large scale sialylation** reaction would be 10-100 fold less than that required for producing the ST6Gal I; the cost of producing the sialyltransferase would be reduced proportionately.

Detail Description Paragraph - DETX (71):

Identification of **Sialyltransferases Useful in Methods for Practical Commercial** Glycoprotein Modification

PGPUB-DOCUMENT-NUMBER: 20030040037

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: **US 20030040037 A1**

TITLE: In vitro modification of glycosylation patterns of recombinant glycopeptides

PUBLICATION-DATE: February 27, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bayer, Robert J.	San Diego	CA	US	

APPL-NO: 10/ 219197

DATE FILED: August 13, 2002

RELATED-US-APPL-DATA:

child 10219197 A1 20020813

parent continuation-of 09855320 20010514 US PENDING

non-provisional-of-provisional 60203851 20000512 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
WO	PCT/US01/15693	2001WO-PCT/US01/15693	May 14, 2001

US-CL-CURRENT: 435/68.1, 435/193 , 435/252.3 , 435/69.1

ABSTRACT:

This invention provides methods for modifying glycosylation patterns of glycopeptides, including recombinantly produced glycopeptides. Also provided are glycopeptide compositions in which the glycopeptides have a uniform glycosylation pattern.

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of prior U.S. provisional application No. 60/203,851, filed May 12, 2000.

---------- KWIC ---------

Pre-Grant Publication Document Identifier - DID (1):
US 20030040037 A1

Detail Description Paragraph - DETX (90):

[0138] In some embodiments, the invention **sialylation methods that have increased commercial practicality through the use of bacterial sialyltransferases,** either recombinantly produced or produced in the native bacterial cells. Two bacterial sialyltransferases have been recently reported; an ST6Gal II from Photobacterium damsela (Yamamoto et al. (1996) J. Biochem. 120: 104-110) and an ST3Gal V from Neisseria meningitidis (Gilbert et al. (1996) J. Biol. Chem. 271: 28271-28276). The two recently described bacterial enzymes transfer sialic acid to the Gal.beta.1,4GlcNAc sequence on oligosaccharide substrates. Table 4 shows the acceptor specificity of these and other sialyltransferases useful in the methods of the invention.

Detail Description Paragraph - DETX (93):

[0141] Other sialyltransferases, including those listed above, are also useful in an economic and efficient large **scale process for sialylation** of commercially important glycopeptides. As described above, a simple test to find out the utility of these other enzymes, is to react various amounts of each enzyme (1-100 mU/mg protein) with a readily available glycopeptide protein such as asialo-.alpha..sub.1-AGP (at 1-10 mg/ml) to compare the ability of the sialyltransferase of interest to sialylate glycopeptides. The results can be compared to, for example, either or both of an ST6Gal I or an ST3Gal III (e.g., a bovine or human enzyme), depending upon the particular sialic acid linkage that is desired. Alternatively, other glycopeptides or glycopeptides, or N- or O-linked oligosaccharides enzymatically released from the peptide backbone can be used in place of asialo-.alpha..sub.1 AGP for this evaluation, or one can use saccharides that are produced by other methods or purified from natural products such as milk. Preferably, however, the sialyltransferases are assayed using an oligosaccharide that is linked to a glycopeptide. Sialyltransferases showing an ability to, for example, sialylate N-linked or O-linked oligosaccharides of glycopeptides more efficiently than ST6Gal I are useful in a practical large **scale process for glycopeptide sialylation**.

PGPUB-DOCUMENT-NUMBER: 20030003529

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: **US 20030003529 A1**

TITLE: Vitro modification of glycosylation patterns of recombinant glycopeptides

PUBLICATION-DATE: January 2, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bayer, Robert J.	San Diego	CA	US	

APPL-NO: 10/ 198806

DATE FILED: July 19, 2002

RELATED-US-APPL-DATA:

child 10198806 A1 20020719

parent division-of 09855320 20010514 US PENDING

non-provisional-of-provisional 60203851 20000512 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
	PCT/US01/15693		May 14, 2001

US-CL-CURRENT: 435/68.1, 435/193 , 435/69.1 , 530/322

ABSTRACT:

This invention provides methods for modifying glycosylation patterns of glycopeptides, including recombinantly produced glycopeptides. Also provided are glycopeptide compositions in which the glycopeptides have a uniform glycosylation pattern.

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of prior U.S. provisional application No. 60/203,851, filed May 12, 2000.

---------- KWIC ---------

Pre-Grant Publication Document Identifier - DID (1):
US 20030003529 A1

Detail Description Paragraph - DETX (90):

[0138] In some embodiments, the invention **sialylation methods that have increased commercial practicality through the use of bacterial sialyltransferases,** either recombinantly produced or produced in the native bacterial cells. Two bacterial sialyltransferases have been recently reported; an ST6Gal II from Photobacterium damsela (Yamamoto et al. (1996) J. Biochem. 120: 104-110) and an ST3Gal V from Neisseria meningitidis (Gilbert et al. (1996) J. Biol. Chem. 271: 28271-28276). The two recently described bacterial enzymes transfer sialic acid to the Gal.beta.1,4GlcNAc sequence on oligosaccharide substrates. Table 4 shows the acceptor specificity of these and other sialyltransferases useful in the methods of the invention.

Detail Description Paragraph - DETX (93):

[0141] Other sialyltransferases, including those listed above, are also useful in an economic and efficient large **scale process for sialylation** of commercially important glycopeptides. As described above, a simple test to find out the utility of these other enzymes, is to react various amounts of each enzyme (1-100 mU/mg protein) with a readily available glycopeptide protein such as asialo-.alpha..sub.1-AGP (at 1-10 mg/ml) to compare the ability of the sialyltransferase of interest to sialylate glycopeptides. The results can be compared to, for example, either or both of an ST6Gal I or an ST3Gal III (e.g., a bovine or human enzyme), depending upon the particular sialic acid linkage that is desired. Alternatively, other glycopeptides or glycopeptides, or N- or O-linked oligosaccharides enzymatically released from the peptide backbone can be used in place of asialo-.alpha..sub.1 AGP for this evaluation, or one can use saccharides that are produced by other methods or purified from natural products such as milk. Preferably, however, the sialyltransferases are assayed using an oligosaccharide that is linked to a glycopeptide. Sialyltransferases showing an ability to, for example, sialylate N-linked or O-linked oligosaccharides of glycopeptides more efficiently than ST6Gal I are useful in a practical large **scale process for glycopeptide sialylation**.

PGPUB-DOCUMENT-NUMBER: 20020160460

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: **US 20020160460 A1**

TITLE: Practical in vitro sialylation of recombinant glycoproteins

PUBLICATION-DATE: October 31, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Paulson, James C.	Del Mar	CA	US	
Bayer, Robert J.	San Diego	CA	US	
Sjoberg, Eric	San Diego	CA	US	

APPL-NO: 10/ 081456

DATE FILED: February 21, 2002

RELATED-US-APPL-DATA:

child 10081456 A1 20020221

parent continuation-of 09007741 19980115 US GRANTED

parent-patent 6399336 US

non-provisional-of-provisional 60035710 19970116 US

US-CL-CURRENT: 435/84, 435/193 , 536/53

ABSTRACT:

This invention provides methods for practical in vitro sialylation of glycoproteins, including recombinantly produced glycoproteins. The methods are useful for large-**scale modification of sialylation** patterns.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application 60/035,710, filed Jan. 16, 1997, which is incorporated herein by reference in its entirety for all purposes.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):
This invention provides methods for practical in vitro sialylation of

glycoproteins, including recombinantly produced glycoproteins. The methods are useful for large-**scale modification of sialylation** patterns.

Pre-Grant Publication Document Identifier - DID (1):
US 20020160460 A1

Detail Description Paragraph - DETX (27):
[0038] The in vitro sialylation methods provided by the invention are, unlike previously described **sialylation methods, practical for commercial**-scale production of modified glycoproteins. Thus, the claimed methods provide a practical means for large-**scale preparation of glycoproteins having altered sialylation** patterns. The methods are well suited for therapeutic glycoproteins that are incompletely sialylated during production in mammalian cells or transgenic animals. The processes provide an increased and consistent level of terminal sialylation of a glycoprotein.

Detail Description Paragraph - DETX (35):
[0046] Other sialyltransferases, including those listed in Table 1, may also be useful in an economic and efficient large **scale process for sialylation** of commercially important glycoproteins. As a simple test to find out the utility of these other enzymes, various amounts of each enzyme (1-100 mU/mg protein) are reacted with asialo-.alpha..sub.1 AGP (at 1-10 mg/ml) to compare the ability of the sialyltransferase of interest to sialylate glycoproteins relative to either bovine ST6Gal I, ST3Gal III or both sialyltransferases. Alternatively, other glycoproteins or glycopeptides, or N-linked oligosaccharides enzymatically released from the peptide backbone can be used in place of asialo-60 .sub.1 AGP for this evaluation. Sialyltransferases showing an ability to sialylate N-linked oligosaccharides of glycoproteins more efficiently than ST6Gal I may prove useful in a practical large **scale process for glycoprotein sialylation** (as illustrated for ST3Gal III in this disclosure).

Detail Description Paragraph - DETX (68):
[0075] This Example demonstrates that sialylation of recombinant glycoproteins using the ST3Gal III sialyltransferase requires much less enzyme than expected. For a one kilogram scale reaction, approximately 7,000 units of the ST3Gal III sialyltransferase would be needed instead of 100,000-150,000 units that the earlier studies indicated. Purification of these enzymes from natural sources is prohibitive, with yields of only 1-10 units for a large scale preparation after 1-2 months work. Assuming that both the ST6Gal I and ST3Gal III sialyltransferases are produced as recombinant sialyltransferases, with equal levels of expression of the two enzymes being achieved, a fermentation scale 14-21 times greater (or more) would be required for the ST6Gal I sialyltransferase relative to the ST3Gal III sialyltransferase. For the ST6Gal I sialyltransferase, expression levels of 0.3 U/1 in yeast has been reported. Borsig et al. (1995) Biochem. Biophys. Res. Commun. 210: 14-20. Expression levels of 1000 U/liter of the ST3Gal III sialyltransferase have been achieved in Aspergillus niger. At current levels of expression 300-450,000 liters of yeast fermentation would be required to produce sufficient enzyme for

sialylation of 1 kg of glycoprotein using the ST6Gal I sialyltransferase. In contrast, less than 10 liter fermentation of Aspergillus niger would be required for sialylation of 1 kg of glycoprotein using the ST3Gal III sialyltransferase. Thus, the fermentation capacity required to produce the ST3Gal III **sialyltransferase for a large scale sialylation** reaction would be 10-100 fold less than that required for producing the ST6Gal I; the cost of producing the sialyltransferase would be reduced proportionately.

Detail Description Paragraph - DETX (80):

Identification of **Sialyltransferases Useful in Methods for Practical Commercial** Glycoprotein Modification

PGPUB-DOCUMENT-NUMBER: 20020150995

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: **US 20020150995 A1**

TITLE: Methods for producing sialyloligosaccharides in a dairy source

PUBLICATION-DATE: October 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Pelletier, Marc	Doylestown	PA	US	
Barker, William A.	West Chester	PA	US	
Hakes, David J.	Willow Grove	PA	US	
Zopf, David A.	Strafford	PA	US	

APPL-NO: 09/ 955909

DATE FILED: September 18, 2001

RELATED-US-APPL-DATA:

child 09955909 A1 20010918

parent continuation-of 08911393 19970814 US GRANTED

parent-patent 6323008 US

US-CL-CURRENT: 435/72

ABSTRACT:

The present invention provides methods for producing sialyloligosaccharides in situ in dairy sources and cheese processing waste streams, prior to, during, or after processing of the dairy source during the cheese manufacturing process. The methods of the present invention use the catalytic activity of .alpha.(2-3) trans-sialidases to exploit the high concentrations of lactose and .alpha.(2-3) sialosides which naturally occur in dairy sources and cheese processing waste streams to drive the enzymatic synthesis of .alpha.(2-3) sialyllactose. .alpha.(2-3) sialyloligosaccharides produced according to these methods are additionally encompassed by the present invention. The invention also provides for recovery of the sialyloligosaccharides produced by these methods. The invention further provides a method for producing .alpha.(2-3) sialyllactose. The invention additionally provides a method of enriching for .alpha.(2-3) sialyllactose in milk using transgenic mammals that express an .alpha.(2-3) trans-sialidase transgene. The invention also provides for recovery of the sialyllactose contained in the milk produced by this transgenic mammal either before or after processing of the milk. Transgenic mammals containing an .alpha.(2-3) trans-sialidase encoding sequence operably linked to a regulatory

sequence of a gene expressed in mammary tissue are also provided by the invention.

---------- KWIC ---------

Pre-Grant Publication Document Identifier - DID (1):
US 20020150995 A1

Summary of Invention Paragraph - BSTX (27):
[0025] Numerous foreign proteins have successfully been transgenically expressed in the milk of livestock. Most of this work has focused on the expression of proteins which are foreign to the mammary gland. Colman, A., 1996, Am. J. Clin. Nutr. 63:639S-645S. To date, milk specific expression of transgenic livestock has been achieved through operably linking regulatory sequences of milk-specific protein genes to the target protein-encoding gene sequence, microinjecting these genetic constructs into the pronuclei of fertilized embryos, and implanting the embryos into recipient females. See e.g. Wright et al., 1991, Biotechnology (NY) 9:830-834; Carver et al., 1993, Biotechnology (NY) 11:1263-1270; Paterson et al., 1994, Appl. Microbiol. Biotechnol. 40:691-698. Proteins that have been successfully expressed in the milk of transgenic animals, include: .alpha.1-antitrypsin (Wright et al., 1991, Biotechnology (NY) 9:830-834; Carver et al., 1993, Biotechnology (NY) 11:1263-1270); Factor IX (Clark et al., 1989, Biotechnology (NY) 7:487-492); protein C (Velander et al., 1992, Proc. Natl. Acad. Sci. USA, 89:12003-12007); tissue plasminogen activator (Ebert et al., 1991, Biotechology (NY) 9:835-838); and fibrinogen. While most of these transgenes express proteins that supplement the composition of milk, very few, if any of the expressed proteins interact directly with the components of milk to alter the natural milk composition. There is a need for methods providing for the large scale production of .alpha.(2-3) sialyloligosaccharides, such as .alpha.(2-3) **sialyllactose, which have commercial** and/or therapeutic valve.

Summary of Invention Paragraph - BSTX (29):
[0026] The present invention greatly advances the field of **commercial production of sialyloligosaccharides** by providing methods for producing sialyloligosaccharides in situ in dairy sources and cheese processing waste streams. The methods of the invention have particular applications in producing .alpha.(2-3) sialyllactose in a dairy source prior to, during, or after processing of the dairy source during the cheese manufacturing process, thereby greatly increasing the recoverable yield of .alpha.(2-3) sialyllactose from the dairy source.

PGPUB-DOCUMENT-NUMBER: 20020142370

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: **US 20020142370 A1**

TITLE: Practical in vitro sialylation of recombinant glycoproteins

PUBLICATION-DATE: October 3, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Paulson, James C.	Del Mar	CA	US	
Bayer, Robert J.	San Diego	CA	US	
Sjoberg, Eric	San Diego	CA	US	

APPL-NO: 10/ 081455

DATE FILED: February 21, 2002

RELATED-US-APPL-DATA:

child 10081455 A1 20020221

parent continuation-of 09007741 19980115 US GRANTED

parent-patent 6399336 US

non-provisional-of-provisional 60035710 19970116 US

US-CL-CURRENT: 435/68.1, 435/200 , 536/53

ABSTRACT:

This invention provides methods for practical in vitro sialylation of glycoproteins, including recombinantly produced glycoproteins. The methods are useful for large-**scale modification of sialylation** patterns.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application 60/035,710, filed Jan. 16, 1997, which is incorporated herein by reference in its entirety for all purposes.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):
This invention provides methods for practical in vitro sialylation of

glycoproteins, including recombinantly produced glycoproteins. The methods are useful for large-**scale modification of sialylation** patterns.

Pre-Grant Publication Document Identifier - DID (1):
US 20020142370 A1

Detail Description Paragraph - DETX (18):
[0030] The in vitro sialylation methods provided by the invention are, unlike previously described **sialylation methods, practical for commercial**-scale production of modified glycoproteins. Thus, the claimed methods provide a practical means for large-**scale preparation of glycoproteins having altered sialylation** patterns. The methods are well suited for therapeutic glycoproteins that are incompletely sialylated during production in mammalian cells or transgenic animals. The processes provide an increased and consistent level of terminal sialylation of a glycoprotein.

Detail Description Paragraph - DETX (26):
[0038] Other sialyltransferases, including those listed in Table 1, may also be useful in an economic and efficient large **scale process for sialylation** of commercially important glycoproteins. As a simple test to find out the utility of these other enzymes, various amounts of each enzyme (1-100 mU/mg protein) are reacted with asialo-.alpha..sub.1 AGP (at 1-10 mg/ml) to compare the ability of the sialyltransferase of interest to sialylate glycoproteins relative to either bovine ST6Gal I, ST3Gal III or both sialyltransferases. Alternatively, other glycoproteins or glycopeptides, or N-linked oligosaccharides enzymatically released from the peptide backbone can be used in place of asialo-.alpha..sub.1 AGP for this evaluation. Sialyltransferases showing an ability to sialylate N-linked oligosaccharides of glycoproteins more efficiently than ST6Gal I may prove useful in a practical large **scale process for glycoprotein sialylation** (as illustrated for ST3Gal III in this disclosure).

Detail Description Paragraph - DETX (59):
[0069] This Example demonstrates that sialylation of recombinant glycoproteins using the ST3Gal III sialyltransferase requires much less enzyme than expected. For a one kilogram scale reaction, approximately 7,000 units of the ST3Gal III sialyltransferase would be needed instead of 100,000-150,000 units that the earlier studies indicated. Purification of these enzymes from natural sources is prohibitive, with yields of only 1-10 units for a large scale preparation after 1-2 months work. Assuming that both the ST6Gal I and ST3Gal III sialyltransferases are produced as recombinant sialyltransferases, with equal levels of expression of the two enzymes being achieved, a fermentation scale 14-21 times greater (or more) would be required for the ST6Gal I sialyltransferase relative to the ST3Gal III sialyltransferase. For the ST6Gal I sialyltransferase, expression levels of 0.3 U/1 in yeast has been reported. Borsig et al. (1995) Biochem. Biophys. Res. Commun. 210: 14-20. Expression levels of 1000 U/liter of the ST3 Gal III sialyltransferase have been achieved in Aspergillus niger. At current levels of expression 300-450,000 liters of yeast fermentation would be required to produce

sufficient enzyme for sialylation of 1 kg of glycoprotein using the ST6Gal I sialyltransferase. In contrast, less than 10 liter fermentation of Aspergillus niger would be required for sialylation of 1 kg of glycoprotein using the ST3Gal III sialyltransferase. Thus, the fermentation capacity required to produce the ST3Gal III **sialyltransferase for a large scale sialylation** reaction would be 10-100 fold less than that required for producing the ST6Gal I; the cost of producing the sialyltransferase would be reduced proportionately.

Detail Description Paragraph - DETX (72):

Identification of **Sialyltransferases Useful in Methods for Practical Commercial** Glycoprotein Modification

PGPUB-DOCUMENT-NUMBER: 20020119516

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: **US 20020119516 A1**

TITLE: Practical in vitro sialylation of recombinant glycoproteins

PUBLICATION-DATE: August 29, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Paulson, James C.	Del Mar	CA	US	
Bayer, Robert J.	San Diego	CA	US	
Sjoberg, Eric	San Diego	CA	US	

APPL-NO: 10/ 007331

DATE FILED: November 9, 2001

RELATED-US-APPL-DATA:

child 10007331 A1 20011109

parent division-of 09007741 19980115 US PENDING

non-provisional-of-provisional 60035710 19970116 US

US-CL-CURRENT: 435/68.1, 435/193

ABSTRACT:

This invention provides methods for practical in vitro sialylation of glycoproteins, including recombinantly produced glycoproteins. The methods are useful for large-**scale modification of sialylation** patterns.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 60/035,710, filed Jan. 16, 1997, which is incorporated herein by reference in its entirety for all purposes.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):

This invention provides methods for practical in vitro sialylation of glycoproteins, including recombinantly produced glycoproteins. The methods are useful for large-**scale modification of sialylation** patterns.

Pre-Grant Publication Document Identifier - DID (1):
US 20020119516 A1

Detail Description Paragraph - DETX (27):
[0040] The in vitro sialylation methods provided by the invention are, unlike previously described **sialylation methods, practical for commercial**-scale production of modified glycoproteins. Thus, the claimed methods provide a practical means for large-**scale preparation of glycoproteins having altered sialylation** patterns. The methods are well suited for therapeutic glycoproteins that are incompletely sialylated during production in mammalian cells or transgenic animals. The processes provide an increased and consistent level of terminal sialylation of a glycoprotein.

Detail Description Paragraph - DETX (38):
[0051] Other sialyltransferases, including those listed in Table 1, may also be useful in an economic and efficient large **scale process for sialylation** of commercially important glycoproteins. As a simple test to find out the utility of these other enzymes, various amounts of each enzyme (1-100 mU/mg protein) are reacted with asialo-.alpha..sub.1 AGP (at 1-10 mg/ml) to compare the ability of the sialyltransferase of interest to sialylate glycoproteins relative to either bovine ST6Gal I, ST3Gal III or both sialyltransferases. Alternatively, other glycoproteins or glycopeptides, or N-linked oligosaccharides enzymatically released from the peptide backbone can be used in place of asialo-.alpha..sub.1 AGP for this evaluation. Sialyltransferases showing an ability to sialylate N-linked oligosaccharides of glycoproteins more efficiently than ST6Gal I may prove useful in a practical large **scale process for glycoprotein sialylation** (as illustrated for ST3Gal III in this disclosure).

Detail Description Paragraph - DETX (71):
[0082] This Example demonstrates that sialylation of recombinant glycoproteins using the ST3 Gal III sialyltransferase requires much less enzyme than expected. For a one kilogram scale reaction, approximately 7,000 units of the ST3Gal III sialyltransferase would be needed instead of 100,000-150,000 units that the earlier studies indicated. Purification of these enzymes from natural sources is prohibitive, with yields of only 1-10 units for a large scale preparation after 1-2 months work. Assuming that both the ST6Gal I and ST3Gal III sialyltransferases are produced as recombinant sialyltransferases, with equal levels of expression of the two enzymes being achieved, a fermentation scale 14-21 times greater (or more) would be required for the ST6Gal I sialyltransferase relative to the ST3Gal III sialyltransferase. For the ST6Gal I sialyltransferase, expression levels of 0.3 U/l in yeast has been reported. Borsig et al. (1995) Biochem. Biophys. Res. Commun. 210: 14-20. Expression levels of 1000 U/liter of the ST3Gal III sialyltransferase have been achieved in Aspergillus niger. At current levels of expression 300-450,000 liters of yeast fermentation would be required to produce sufficient enzyme for sialylation of 1 kg of glycoprotein using the ST6Gal I sialyltransferase. In contrast, less than 10 liter fermentation of Aspergillus niger would be

required for sialylation of 1 kg of glycoprotein using the ST3Gal III sialyltransferase. Thus, the fermentation capacity required to produce the ST3 Gal III **sialyltransferase for a large scale sialylation** reaction would be 10-100 fold less than that required for producing the ST6Gal I; the cost of producing the sialyltransferase would be reduced proportionately.

Detail Description Paragraph - DETX (84):

Identification of **Sialyltransferases Useful in Methods For Practical Commercial** Glycoprotein Modification

PGPUB-DOCUMENT-NUMBER: 20020068331

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: **US 20020068331 A1**

TITLE: Production of fucosylated carbohydrates by enzymatic fucosylation synthesis of sugar nucleotides; and in situ regeneration of GDP-fucose

PUBLICATION-DATE: June 6, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Wong, Chi-Huey	Rancho Santa Fe	CA	US	
Ichikawa, Yoshitaka	San Diego	CA	US	
Shen, Gwo-Jenn	Carlsbad	CA	US	
Liu, Kun-Chin	New Haven	CT	US	

APPL-NO: 09/ 992680

DATE FILED: November 19, 2001

RELATED-US-APPL-DATA:

child 09992680 A1 20011119

parent division-of 07961076 19921014 US GRANTED

parent-patent 6319695 US

child 07961076 19921014 US

parent continuation-in-part-of 07910612 19920708 US ABANDONED

child 07910612 19920708 US

parent continuation-in-part-of 07901260 19920619 US ABANDONED

child 07901260 19920619 US

parent continuation-in-part-of 07777662 19911015 US ABANDONED

US-CL-CURRENT: 435/74, 435/72

ABSTRACT:

This invention contemplates improved methods of enzymatic production of carbohydrates especially fucosylated carbohydrates. Improved syntheses of glycosyl 1- or 2-phosphates using both chemical and enzymatic means are also contemplated. The phosphorylated glycosides are then used to produce sugar

nucleotides that are in turn used as donor sugars for glycosylation of acceptor carbohydrates. Especially preferred herein is the use of a disclosed method for fucosylation.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 07/910,612, filed Jul. 8, 1992, that is a continuation-in-part of U.S. patent application Ser. No. 07/901,260, filed Jun. 19, 1992, that is a continuation-in-part of U.S. patent application Ser. No. 07/777,662, filed Oct. 15, 1991, now abandoned, whose disclosures are incorporated herein by reference.

---------- KWIC ---------

Pre-Grant Publication Document Identifier - DID (1):
US 20020068331 A1

Detail Description Paragraph - DETX (84):
[0159] An alternative method was to start with Fuc-1-P, which was converted to GDP-Fuc catalyzed by GDP-Fuc pyrophosphorylase (GDP-Fuc P), as shown in Scheme 20, below). [Ishihara et al., J. Biol. Chem., 243:1103 (1968); Ishihara et al., J. Biol. Chem., 243:1110 (1968); Schachter et al., Methods in Enzymol., 28:285 (1972); Richards et al., Biochim. Biophys. Acta, 484:353 (1977); Kilker et al., Biochim. Biophys. Acta, 570:271 (1979)]. GDP-Fuc P has been partially purified from porcine liver [Ishihara et al., J. Biol. Chem., 243:1110 (1968)] and it has been demonstrated that the regeneration system depicted in Scheme 20 is functional on an analytical **scale for the synthesis of Le.sup.x and sialyl** Le.sup.x. 26

PGPUB-DOCUMENT-NUMBER: 20020034805

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: **US 20020034805 A1**

TITLE: FUSION PROTEINS FOR USE IN ENZYMATIC SYNTHYESIS OF OLIGOSACCHARIDES

PUBLICATION-DATE: March 21, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
GILBERT, MICHEL	HULL		CA	
YOUNG, N. MARTIN	GLOUCESTER		CA	
WAKARCHUK, WARREN W.	GLOUCESTER		CA	

APPL-NO: 09/ 211691

DATE FILED: December 14, 1998

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60069443 19971215 US

US-CL-CURRENT: 435/193, 435/183 , 435/200 , 435/320.1 , 435/325 , 536/23.2

ABSTRACT:

This invention provides fusion polypeptides that include a glycosyltransferase catalytic domain and a catalytic domain from an accessory enzyme that is involved in making a substrate for a glycosyltransferase reaction. Nucleic acids that encode the fusion polypeptides are also provided, as are host cells for expressing the fusion polypeptides of the invention.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of, and claims benefit of, US Provisional Application No. 60/069,443, filed Dec. 15, 1997, which application is incorporated herein by reference for all purposes.

---------- KWIC ---------

Pre-Grant Publication Document Identifier - DID (1):
US 20020034805 A1

Detail Description Paragraph - DETX (127):

[0136] This Example describes the construction and expression of a polynucleotide that encodes a fusion protein that has both CMP-Neu5Ac synthetase activity and .alpha.2,3-sialyltransferase activity. Large-scale enzymatic synthesis of oligosaccharides containing terminal N-acetyl-neuraminic acid residues requires large amounts of the sialyltransferase and the corresponding sugar-nucleotide synthetase for the synthesis of the sugar-nucleotide donor, CMP-Neu5Ac, an unstable compound. Using genes cloned from Neisseria meningitidis, we constructed a fusion protein which has both CMP-Neu5Ac synthetase and cc-2,3-sialyltransferase activities. The fusion protein was produced in high yields (over 1,200 units per liter, measured using an .alpha.-2,3-sialyltransferase assay) in Escherichia coli and functionally pure enzyme could be obtained using a simple protocol. In small-scale enzymatic syntheses, we showed that the fusion protein could sialylate various oligosaccharide acceptors (branched and linear) with N-acetyl-neuraminic acid as well as N-glycolyl- and N-propionyl-neuraminic acid in high conversion yield. The fusion protein was also used to produce .alpha.-2,3-**sialyllactose at the 100 g scale** using a sugar nucleotide cycle reaction, starting from lactose, sialic acid, phosphoenolpyruvate and catalytic amounts of ATP and CMP.

Detail Description Paragraph - DETX (150):

[0159] E. coli BMH71-18 was transformed with the three versions of pFUS-01 and the level of .alpha.-2,3-**sialyltransferase activity was compared in small-scale** cultures (20 mL). The highest activity was obtained with pFUS-01/2, which gave 40% more activity than pFUS-01/4 and 60% more activity than pFUS-01. The fusion protein encoded by pFUS-01/2 has the longest linker which might aid the independent folding of the two components. However, the effects of linker composition and length were not further studied and pFUS-01/2 was used for the scale-up in production and kinetics comparison.

Detail Description Paragraph - DETX (166):

[0175] To be useful for large scale carbohydrate synthesis the fusion protein should be applicable in a sugar nucleotide cycle. This cycle is designed to use only catalytic amounts of expensive sugar nucleotides and nucleoside phosphates, which are enzymatically regenerated in situ from low-cost precursors. The recycling of the converted co-factors also prevents end-product inhibition. The .alpha.-2,3-**sialyllactose 100 g scale** synthesis went to completion, which is important since stoichiometric conversion of substrates is desirable not only to minimize reagent costs but also because it greatly simplifies the purification of the product from a large scale synthesis. Another interesting feature of the fusion protein is that it can use directly different donor analogs and various acceptors with a terminal galactose residue. Consequently it can be used for the synthesis of both natural carbohydrates and synthetic derivatives with novel properties.

PGPUB-DOCUMENT-NUMBER: 20020019342

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: **US 20020019342 A1**

TITLE: In vitro modification of glycosylation patterns of recombinant glycopeptides

PUBLICATION-DATE: February 14, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bayer, Robert	San Diego	CA	US	

APPL-NO: 09/ 855320

DATE FILED: May 14, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60203851 20000512 US

US-CL-CURRENT: 514/8, 435/14

ABSTRACT:

This invention provides methods for modifying glycosylation patterns of glycopeptides, including recombinantly produced glycopeptides. Also provided are glycopeptide compositions in which the glycopeptides have a uniform glycosylation pattern.

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of prior U.S. provisional application number 60/203,851, filed May 12, 2000.

---------- KWIC ---------

Pre-Grant Publication Document Identifier - DID (1):
US 20020019342 A1

Detail Description Paragraph - DETX (90):
[0138] In some embodiments, the invention **sialylation methods that have increased commercial practicality through the use of bacterial sialyltransferases,** either recombinantly produced or produced in the native bacterial cells. Two bacterial sialyltransferases have been recently reported; an ST6Gal II from Photobacterium damsela (Yamamoto et al. (1996) J. Biochem.

120: 104-110) and an ST3Gal V from Neisseria meningitidis (Gilbert et al. (1996) J. Biol. Chem. 271: 28271-28276). The two recently described bacterial enzymes transfer sialic acid to the Gal.beta.1,4GlcNAc sequence on oligosaccharide substrates. Table 4 shows the acceptor specificity of these and other sialyltransferases useful in the methods of the invention.

Detail Description Paragraph - DETX (93):

[0141] Other sialyltransferases, including those listed above, are also useful in an economic and efficient large **scale process for sialylation** of commercially important glycopeptides. As described above, a simple test to find out the utility of these other enzymes, is to react various amounts of each enzyme (1-100 mU/mg protein) with a readily available glycopeptide protein such as asialo-.alpha..sub.1-AGP (at 1-10 mg/ml) to compare the ability of the sialyltransferase of interest to sialylate glycopeptides. The results can be compared to, for example, either or both of an ST6Gal I or an ST3Gal III (e.g., a bovine or human enzyme), depending upon the particular sialic acid linkage that is desired. Alternatively, other glycopeptides or glycopeptides, or N- or O-linked oligosaccharides enzymatically released from the peptide backbone can be used in place of asialo-.alpha..sub.1 AGP for this evaluation, or one can use saccharides that are produced by other methods or purified from natural products such as milk. Preferably, however, the sialyltransferases are assayed using an oligosaccharide that is linked to a glycopeptide. Sialyltransferases showing an ability to, for example, sialylate N-linked or O-linked oligosaccharides of glycopeptides more efficiently than ST6Gal I are useful in a practical large **scale process for glycopeptide sialylation**.

US-PAT-NO: **6596523**

DOCUMENT-IDENTIFIER: US 6596523 B1

TITLE: .alpha.,2,8-sialyltransferase

DATE-ISSUED: July 22, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sasaki; Katsutoshi	Tokyo	N/A	N/A	JP
Miura; Kazumi	Kanagawa	N/A	N/A	JP
Hanai; Nobuo	Kanagawa	N/A	N/A	JP
Nishi; Tatsunari	Tokyo	N/A	N/A	JP

APPL-NO: 08/ 361304

DATE FILED: November 29, 1994

PARENT-CASE:

This application is a continuation-in-part of PCT/JP94/00495, filed Mar. 28, 1994 designating the U.S.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	5-069988	March 29, 1993

US-CL-CURRENT: 435/193, 536/23.2

ABSTRACT:

The invention provides a novel .alpha.-2,8-sialyltransferase expressed by a gene cloned from animal cells, a cDNA coding for the .alpha.-2,8-sialyltransferase, a method of detecting, or suppressing the production of .alpha.-2,8-sialyltransferase by using the cDNA, a recombinant vector containing the DNA as an insert and cells harboring the recombinant vector as well as methods of preparing same. The .alpha.-2,8-sialyltransferase of the invention is useful, for example, in the production of carbohydrate chains having a useful physiological activity, for example the ganglioside GD3, and modifications thereof.

1 Claims, 24 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 24

---------- KWIC ---------

US Patent No. - PN (1):
6596523

Brief Summary Text - BSTX (22):

As far as sialyltransferase is concerned, a gene for an enzyme having .beta.-galactoside .alpha.-2,6-sialyltransferase activity has been isolated and the base sequence thereof has been reported [Weinstein et al.: Journal of Biological Chemistry, 262, 17735 (1987)]. As regards an enzyme having .beta.-galactoside .alpha.-2,3-sialyltransferase activity, cloning of a gene coding for an enzyme catalyzing the addition of sialic acid to galactose in an O-glycoside bond type carbohydrate chain (carbohydrate chain added to a serine or threonine residue) of glycoproteins has been reported by Gillespie et al. but the base sequence of said gene has not been reported [Gillespie et al.: Glycoconjugate Journal, 7, 469 (1990)]. Weinstein et al. reported a method of purifying an enzyme having .beta.-galactoside .alpha.-2,3-sialyltransferase activity from rat liver [Weinstein et al.: Journal of Biological Chemistry, 257, 13835 (1982)]. This method, however, provides the desired enzyme only in very small amounts. This rat liver .beta.-galactoside .alpha.-2,3-sialyltransferase gene has been cloned by Wen et al. [Wen et al.: Journal of Biological Chemistry, 267, 21011 (1992)]. There has been no report, however, of the cloning of a gene for human galactoside .alpha.-2,8-sialyltransferase. Large **scale preparation of a sialyltransferase** species having .alpha.-2,8-sialyltransferase activity or cloning of a gene for encoding a product having sialyltransferase activity has not been reported as yet. Therefore, no means is currently available for large **scale preparation of a sialyltransferase** having .alpha.-2,8-sialyltransferase activity, in particular human galactoside .alpha.-2,8-sialyltransferase. Methods of detecting or suppressing expression of the enzyme have also not been established.

US-PAT-NO: **6500661**

DOCUMENT-IDENTIFIER: US 6500661 B1

TITLE: Enzymatic conversion of GDP-mannose to GDP-fucose

DATE-ISSUED: December 31, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sjoberg; Eric R.	San Diego	CA	N/A	N/A

APPL-NO: 09/ 231905

DATE FILED: January 14, 1999

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of U.S. Provisional Application No. 60/071,076, filed Jan. 15, 1998, which application is incorporated herein by reference for all purposes.

US-CL-CURRENT: 435/233, 435/183 , 435/189 , 435/191 , 435/194 , 435/195

ABSTRACT:

This invention provides methods for practical enzymatic conversion of GDP-mannose to GDP-fucose. These methods are useful for efficient synthesis of reactants used in the synthesis of fucosylated oligosaccharides.

20 Claims, 16 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

---------- KWIC ---------

US Patent No. - PN (1):
6500661

Drawing Description Text - DRTX (13):
FIG. 12 shows a time course of a large**-scale sialyl** Lewis X synthesis reaction. Samples were taken from the reaction mixture and analyzed by TLC. From left to right, the lanes are as follows: Lane 1, reaction at 160 hours;

Lane 2, 147 hours; Lane 3, SLN; Lane 4, 43 hours; Lane 5, 1 hour.

Detailed Description Text - DETX (186):

To demonstrate the utility of bacterial GDP-mannose dehydratase and YEF B for the large **scale synthesis of sialyl** Lewis X antigen and other bioactive carbohydrates, a 100 gram reaction was conducted in which Neu5Ac.alpha.2,3Gal.beta.1,4GlcNAc.beta.1,3Gal-OR (SLN), the tetrasaccharide precursor of sialyl Lewis X antigen, was fucosylated to form sialyl Lewis X antigen. Conversion of SLN into sialyl Lewis X antigen was monitored by HPLC, TLC and pH by pH electrode. The results of this assay are shown in FIG. 10, in which formation of sialyl Lewis X is monitored by detecting the increase in free GDP concentration. The pH of the reaction mixture is also shown. As GDP was hydrolyzed by alkaline phosphatase liberating inorganic phosphate, the pH of the reaction decreased. The liberated inorganic phosphate also formed a precipitate with magnesium ions, decreasing the concentration of free magnesium, as shown in FIG. 11.

US-PAT-NO: **6399336**

DOCUMENT-IDENTIFIER: US 6399336 B1

TITLE: Practical in vitro sialylation of recombinant glycoproteins

DATE-ISSUED: June 4, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Paulson; James C.	Del Mar	CA	N/A	N/A
Bayer; Robert J.	San Diego	CA	N/A	N/A
Sjoberg; Eric	San Diego	CA	N/A	N/A

APPL-NO: 09/ 007741

DATE FILED: January 15, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 60/035,710, filed Jan. 16, 1997, which is incorporated herein by reference in its entirety for all purposes.

US-CL-CURRENT: 435/97, 435/15 , 435/183 , 435/193 , 435/220 , 435/252.3 , 435/4 , 435/41 , 435/7.2

ABSTRACT:

This invention provides methods for practical in vitro sialylation of glycoproteins, including recombinantly produced glycoproteins. The methods are useful for large-**scale modification of sialylation** patterns.

87 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

---------- KWIC ---------

Abstract Text - ABTX (1):

This invention provides methods for practical in vitro sialylation of glycoproteins, including recombinantly produced glycoproteins. The methods are useful for large-**scale modification of sialylation** patterns.

US Patent No. - PN (1):
6399336

Detailed Description Text - DETX (27):
The in vitro sialylation methods provided by the invention are, unlike previously described **sialylation methods, practical for commercial**-scale production of modified glycoproteins. Thus, the claimed methods provide a practical means for large-**scale preparation of glycoproteins having altered sialylation** patterns. The methods are well suited for therapeutic glycoproteins that are incompletely sialylated during production in mammalian cells or transgenic animals. The processes provide an increased and consistent level of terminal sialylation of a glycoprotein.

Detailed Description Text - DETX (38):
Other sialyltransferases, including those listed in Table 1, may also be useful in an economic and efficient large **scale process for sialylation** of commercially important glycoproteins. As a simple test to find out the utility of these other enzymes, various amounts of each enzyme (1-100 mU/mg protein) are reacted with asialo-.alpha..sub.1 AGP (at 1-10 mg/ml) to compare the ability of the sialyltransferase of interest to sialylate glycoproteins relative to either bovine ST6Gal I, ST3Gal III or both sialyltransferases. Alternatively, other glycoproteins or glycopeptides, or N-linked oligosaccharides enzymatically released from the peptide backbone can be used in place of asialo-.alpha..sub.1 AGP for this evaluation. Sialyltransferases showing an ability to sialylate N-linked oligosaccharides of glycoproteins more efficiently than ST6Gal I may prove useful in a practical large **scale process for glycoprotein sialylation** (as illustrated for ST3Gal III in this disclosure).

Detailed Description Text - DETX (71):
This Example demonstrates that sialylation of recombinant glycoproteins using the ST3 Gal III sialyltransferase requires much less enzyme than expected. For a one kilogram scale reaction, approximately 7,000 units of the ST3Gal III sialyltransferase would be needed instead of 100,000-150,000 units that the earlier studies indicated. Purification of these enzymes from natural sources is prohibitive, with yields of only 1-10 units for a large scale preparation after 1-2 months work. Assuming that both the ST6Gal I and ST3Gal III sialyltransferases are produced as recombinant sialyltransferases, with equal levels of expression of the two enzymes being achieved, a fermentation scale 14-21 times greater (or more) would be required for the ST6Gal I sialyltransferase relative to the ST3Gal III sialyltransferase. For the ST6Gal I sialyltransferase, expression levels of 0.3 U/1 in yeast has been reported. Borsig et al. (1995) Biochem. Biophys. Res. Commun. 210: 14-20. Expression levels of 1000 U/liter of the ST3 Gal III sialyltransferase have been achieved in Aspergillus niger. At current levels of expression 300-450,000 liters of yeast fermentation would be required to produce sufficient enzyme for sialylation of 1 kg of glycoprotein using the ST6Gal I sialyltransferase. In contrast, less than 10 liter fermentation of Aspergillus niger would be

required for sialylation of 1 kg of glycoprotein using the ST3Gal III sialyltransferase. Thus, the fermentation capacity required to produce the ST3Gal III **sialyltransferase for a large scale sialylation** reaction would be 10-100 fold less than that required for producing the ST6Gal I; the cost of producing the sialyltransferase would be reduced proportionately.

Detailed Description Text - DETX (84):

Identification of **Sialyltransferases Useful in Methods for Practical Commercial** Glycoprotein Modification

Claims Text - CLTX (1):

1. A large-**scale method of sialylating** a saccharide group on a recombinant glycoprotein, the method comprising contacting a saccharide group which comprises a galactose or N-acetylgalactosamine acceptor moiety on a recombinant glycoprotein with a sialic acid donor moiety and a recombinant sialyltransferase in a reaction mixture which provides reactants required for sialyltransferase activity for a sufficient time and under appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said saccharide group wherein at least about 80% of the saccharide groups are sialylated, and wherein said recombinant sialyltransferase is present at 50 mU or less per mg of said recombinant glycoprotein.

Claims Text - CLTX (23):

23. A large-**scale method of sialylating** a saccharide group on a recombinant glycoprotein, the method comprising contacting a saccharide group which comprises a galactose or an N-acetylgalactosamine acceptor moiety on a recombinant glycoprotein with a sialic acid donor moiety and a bacterial sialyltransferase in a reaction mixture which provides reactants required for sialyltransferase activity for a sufficient time and under appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said saccharide group and wherein at least about 80% of the saccharide groups are sialylated, wherein said bacterial sialyltransferase is present at 50 mU or less per mg of said recombinant glycoprotein.

Claims Text - CLTX (32):

32. A large-**scale method for in vitro sialylation** of saccharide group s present on a glycoprotein, said method comprising contacting said saccharide groups with a sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase activity for a sufficient time and under appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said saccharide group, wherein said sialyltransferase is present at a concentration about 50 mU per mg of glycoprotein or less, and wherein at least about 80% of the saccharide groups are sialylated.

Claims Text - CLTX (57):

57. A large-**scale method for in vitro sialylation** of terminal galactose residues present on a glycoprotein, said method comprising contacting said glycoprotein with a reaction mixture that comprises a sialyltransferase, a

sialic acid donor moiety, and other reactants required for sialyltransferase activity, for a sufficient time and under appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said terminal galactose residues, wherein at least about 80% of the terminal galactose residues present on the glycoprotein are sialylated, and wherein said sialyltransferase is present at a concentration about 50 mU per mg of glycoprotein or less.

Claims Text - CLTX (59):

59. A large-**scale method for in vitro sialylation** of terminal galactose residues present on a glycoprotein, said method comprising contacting said glycoprotein with a reaction mixture that comprises a sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase activity, for a sufficient time and under appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said terminal galactose residues, wherein a greater percentage of terminal galactose residues are sialylated compared to an unaltered glycoprotein, wherein said greater percentage is equal to at least about 80%, and wherein said sialyltransferase is present at a concentration about 50 mU per mg of glycoprotein or less.

US-PAT-NO: **6323008**

DOCUMENT-IDENTIFIER: US 6323008 B1

TITLE: . Methods for producing sialyloligosaccharides in a dairy source

DATE-ISSUED: November 27, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Pelletier; Marc	Doylestown	PA	N/A	N/A
Barker; William A.	West Chester	PA	N/A	N/A
Hakes; David J.	Willow Grove	PA	N/A	N/A
Zopf; David A.	Strafford	PA	N/A	N/A

APPL-NO: 08/ 911393

DATE FILED: August 14, 1997

US-CL-CURRENT: 435/84, 435/101, 435/274 , 435/99 , 536/124 , 536/127

ABSTRACT:

The present invention provides methods for producing sialyloligosaccharides in situ in dairy sources and cheese processing waste streams, prior to, during, or after processing of the dairy source during the cheese manufacturing process. The methods of the present invention use the catalytic activity of .alpha.(2-3) trans-sialidases to exploit the high concentrations of lactose and .alpha.(2-3) sialosides which naturally occur in dairy sources and cheese processing waste streams to drive the enzymatic synthesis of .alpha.(2-3) sialyllactose. .alpha.(2-3) sialyloligosaccharides produced according to these methods are additionally encompassed by the present invention. The invention also provides for recovery of the sialyloligosaccharides produced by these methods. The invention further provides a method for producing .alpha.(2-3) sialyllactose. The invention additionally provides a method of enriching for .alpha.(2-3) sialyllactose in milk using transgenic mammals that express an .alpha.(2-3) trans-sialidase transgene. The invention also provides for recovery of the sialyllactose contained in the milk produced by this transgenic mammal either before or after processing of the milk. Transgenic mammals containing an .alpha.(2-3) trans-sialidase encoding sequence operably linked to a regulatory sequence of a gene expressed in mammary tissue are also provided by the invention.

35 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

---------- KWIC ---------

US Patent No. - PN (1):
6323008

Brief Summary Text - BSTX (27):
Numerous foreign proteins have successfully been transgenically expressed in the milk of livestock. Most of this work has focused on the expression of proteins which are foreign to the mammary gland. Colman, A., 1996, Am. J. Clin. Nutr. 63:639S-645S. To date, milk specific expression of transgenic livestock has been achieved through operably linking regulatory sequences of milk-specific protein genes to the target protein-encoding gene sequence, microinjecting these genetic constructs into the pronuclei of fertilized embryos, and implanting the embryos into recipient females. See e.g. Wright et al., 1991, Biotechnology (NY) 9:830-834; Carver et al., 1993,.Biotechnology (NY) 11:1263-1270; Paterson et al., 1994, Appl. Microbiol. Biotechnol. 40:691-698. Proteins that have been successfully expressed in the milk of transgenic animals, include: .alpha.1-antitrypsin (Wright et al., 1991, Biotechnology (NY) 9:830-834; Carver et al., 1993, Biotechnology (NY) 11:1263-1270); Factor IX (Clark et al., 1989, Biotechnology (NY) 7:487-492); protein C (Velander et al., 1992, Proc. Natl. Acad. Sci. USA, 89:12003-12007); tissue plasminogen activator (Ebert et al., 1991, Biotechology (NY) 9:835-838); and fibrinogen. While most of these transgenes express proteins that supplement the composition of milk, very few, if any of the expressed proteins interact directly with the components of milk to alter the natural milk composition. There is a need for methods providing for the large scale production of .alpha.(2-3) sialyloligosaccharides, such as .alpha.(2-3) **sialyllactose, which have commercial** and/or therapeutic valve.

Brief Summary Text - BSTX (29):
The present invention greatly advances the field of **commercial production of sialyloligosaccharides** by providing methods for producing sialyloligosaccharides in situ in dairy sources and cheese processing waste streams. The methods of the invention have particular applications in producing .alpha.(2-3) sialyllactose in a dairy source prior to, during, or after processing of the dairy source during the cheese manufacturing process, thereby greatly increasing the recoverable yield of .alpha.(2-3) sialyllactose from the dairy source.

US-PAT-NO: **6319695**

DOCUMENT-IDENTIFIER: US 6319695 B1

TITLE: Production of fucosylated carbohydrates by enzymatic fucosylation synthesis of sugar nucleotides; and in situ regeneration of GDP-fucose

DATE-ISSUED: November 20, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wong; Chi-Huey	Rancho Santa Fe	CA	N/A	N/A
Ichikawa; Yoshitaka	San Diego	CA	N/A	N/A
Shen; Gwo-Jenn	Carlsbad	CA	N/A	N/A
Liu; Kun-Chin	New Haven	CT	N/A	N/A

APPL-NO: 07/ 961076

DATE FILED: October 14, 1992

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. patent application Ser. No. 07/910,612, filed Jul. 8, 1992, now abandoned, that is a continuation-in-part of U.S. patent application Ser. No. 07/901,260, filed Jun. 19, 1992, now abandoned, that is a continuation-in-part of U.S. patent application Ser. No. 07/777,662, filed Oct. 15, 1991, now abandoned, whose disclosures are incorporated herein by reference.

US-CL-CURRENT: 435/97, 435/84

ABSTRACT:

This invention contemplates improved methods of enzymatic production of carbohydrates especially fucosylated carbohydrates. Improved syntheses of glycosyl 1- or 2-phosphates using both chemical and enzymatic means are also contemplated. The phosphorylated glycosides are then used to produce sugar nucleotides that are in turn used as donor sugars for glycosylation of acceptor carbohydrates. Especially preferred herein is the use of a disclosed method for fucosylation.

20 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

---------- KWIC ---------

US Patent No. - PN (1):
6319695

Detailed Description Text - DETX (83):
An alternative method was to start with Fuc-1-P, which was converted to GDP-Fuc catalyzed by GDP-Fuc pyrophosphorylase (GDP-Fuc P), as shown in Scheme 20, below). [Ishihara et al., J. Biol. Chem., 243:1103 (1968); Ishihara et al., J. Biol. Chem., 243:1110 (1968); Schachter et al., Methods in Enzymol., 28:285 (1972); Richards et al., Biochim. Biophys. Acta, 484:353 (1977); Kilker et al., Biochim. Biophys. Acta, 570:271 (1979)]. GDP-Fuc P has been partially purified from porcine liver [Ishihara et al., J. Biol. Chem., 243:1110 (1968)] and it has been demonstrated that the regeneration system depicted in Scheme 20 is functional on an analytical **scale for the synthesis of Le.sup.x and sialyl** Le.sup.x. ##STR26##

US-PAT-NO: **6168934**

DOCUMENT-IDENTIFIER: US 6168934 B1

TITLE: Oligosaccharide enzyme substrates and inhibitors: methods and compositions

DATE-ISSUED: January 2, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wong; Chi-Huey	San Diego	CA	N/A	N/A
Ichikawa; Yoshitaka	San Diego	CA	N/A	N/A
Shen; Gwo-Jenn	Carlsbad	CA	N/A	N/A

APPL-NO: 09/ 072958

DATE FILED: May 5, 1998

PARENT-CASE:

CROSS-REFERENCE TO COPENDING APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 08/472,877 filed Jun. 7, 1995, now U.S. Pat. No. 5,759,823, which is itself a division of U.S. patent application Ser. No. 08/219,242 filed Mar. 29, 1994, now U.S. Pat. No. 5,461,143, which is a continuation-in-part of U.S. patent application Ser. No. 07/852,409, filed Mar. 16, 1992, now abandoned, which is itself a continuation-in-part of U.S. patent application Ser. No. 07/738,211 filed Jul. 30, 1991, now abandoned, which is itself a continuation-in-part of U.S. patent application Ser. No. 07/670,701 filed Mar. 18, 1991, now U.S. Pat. No. 5,278,299, and U.S. patent application Ser. No. 07/707,600 filed May 30, 1991, now abandoned, the benefit of whose filing dates the present application is entitled abandoned.

US-CL-CURRENT: 435/97, 435/100 , 435/101 , 435/183 , 435/74 , 435/85

ABSTRACT:

Oligosaccharide compounds that are substrates and inhibitors of glycosyltransferase and glycosidase enzymes and compositions containing such compounds are disclosed. A method of glycosylation is also disclosed. An E. coli transformed with phagemid CMPSIL-1, which phagemid comprises a gene for a modified CMP-sialic acid synthetase enzyme, which transformed E. coli has the ATCC accession No. 68531 is also provided.

11 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

---------- KWIC ---------

US Patent No. - PN (1):
6168934

Detailed Description Text - DETX (250):
These data show that a glycosyl compound can be sialylated in an efficient, enzyme-catalyzed, self-contained, cyclic, synthetic method involving the in situ regeneration of CMP-sialic acid. This synthetic method provides a novel, high-yield (97 percent) scheme for the large-**scale preparation of sialylated** glycosyl compounds.

US-PAT-NO: **5834251**

DOCUMENT-IDENTIFIER: US 5834251 A

TITLE: Methods of modifying carbohydrate moieties

DATE-ISSUED: November 10, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Maras; Marleen	Gentbrugge	N/A	N/A	BE
Contreras; Roland	Merelbeke	N/A	N/A	BE

APPL-NO: 08/ 366800

DATE FILED: December 30, 1994

US-CL-CURRENT: 435/71.1, 435/171 , 435/68.1 , 435/69.1 , 435/72 , 435/85 , 435/97 , 435/99

ABSTRACT:

The invention is directed to methods of converting high mannose type glycosylation patterns to hybrid or complex type glycosylation patterns.

20 Claims, 16 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 16

---------- KWIC ---------

US Patent No. - PN (1):
5834251

Detailed Description Text - DETX (136):
Additional in vitro modifications of GlcNAc-bearing glycoproteins or oligosaccharides were carried out with the commercial .beta.-1,4 galactosyl transferase (Boehringer Mannheim) from human milk (Schanbacher et al., J. Biol. Chem. 245:5057-5061 (1970)) and **commercial .alpha.-2,6 sialyltransferase** (Boehringer Mannheim) from rat liver (Weinstein et al., J. Biol. Chem. 257:13845-13853 (1982)). Galactosylation was demonstrated through the incorporation of radioactive galactose on in vitro synthesized, non-labelled, GlcNAc-bearing glycosylstructures. The completion of the synthetic pathway was demonstrated through the incorporation of radioactive sialic acid on "cold" galactose that was added in a .beta.-1,4 linkage to GlcNAc. Glycoproteins

labelled in this manner were separated through electrophoresis on a SDS-polyacrylamide gel. During electrophoresis, sialylated glycoproteins moved slower compared to non-sialylated proteins. This was observed as a shift of the sialylated proteins towards higher molecular weights. To clearly demonstrate this effect, one Trichoderma protein, namely cellobiohydrolase I (CBH I), was examined in particular.

US-PAT-NO: **5759823**

DOCUMENT-IDENTIFIER: US 5759823 A
See image for Certificate of Correction

TITLE: Ogligosaccharide enzyme substrates and inhibitors: methods and compostions

DATE-ISSUED: June 2, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wong; Chi-Huey	San Diego	CA	N/A	N/A
Ichikawa; Yoshitaka	San Diego	CA	N/A	N/A
Shen; Gwo-Jenn	Carlsbad	CA	N/A	N/A

APPL-NO: 08/ 472877

DATE FILED: June 7, 1995

PARENT-CASE:

CROSS-REFERENCE TO COPENDING APPLICATIONS

This is a division of application Ser. No. 08/219,242, filed Mar. 29, 1994, now U.S. Pat. No. 5,461,143, which is a continuation-in-part of U.S. patent application Ser. No. 07/852,409, filed Mar. 16, 1992, now abandoned, which is itself a continuation-in-part of U.S. patent application Ser. No. 07/738,211 filed Jul. 30, 1991, now abandoned, which is itself a continuation-in-part of U.S. patent application Ser. No. 07/670,701 filed Mar. 18, 1991, now U.S. Pat. No. 5,278,299, and U.S. patent application Ser. No. 07/707,600 filed May 30, 1991, now abandoned.

US-CL-CURRENT: 435/97, 435/100 , 435/101 , 435/74 , 435/85

ABSTRACT:

Oligosaccharide compounds that are substrates and inhibitors of glycosyltransferase and glycosidase enzymes and compositions containing such compounds are disclosed. A method of glycosylation is also disclosed. An E. coli transformed with phagemid CMPSIL-1, which phagemid comprises a gene for a modified CMP-sialic acid synthetase enzyme, which transformed E. coli has the ATCC accession No. 68531 is also provided.

24 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

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US Patent No. - PN (1):
5759823

Detailed Description Text - DETX (299):
These data show that a glycosyl compound can be sialylated in an efficient, enzyme-catalyzed, self-contained, cyclic, synthetic method involving the in situ regeneration of CMP-sialic acid. This synthetic method provides a novel, high-yield (97 percent) scheme for the large-**scale preparation of sialylated** glycosyl compounds.

US-PAT-NO: **5593887**

DOCUMENT-IDENTIFIER: US 5593887 A
See image for Certificate of Correction

TITLE: Oligosaccharide enzyme substrates and inhibitors: methods and compositions

DATE-ISSUED: January 14, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wong; Chi-Huey	San Diego	CA	N/A	N/A
Ichikawa; Yoshitaka	San Diego	CA	N/A	N/A
Shen; Gwo-Jenn	Carlsbad	CA	N/A	N/A

APPL-NO: 08/ 476685

DATE FILED: June 7, 1995

PARENT-CASE:

CROSS-REFERENCE TO COPENDING APPLICATIONS

This application is a divisional of U.S. patent application Ser. No. 08/219,242, filed Mar 29, 1994, now U.S. Pat. No. 5,461,143, which is a continuation-in-part of U.S. patent application Ser. No. 07/852,409, filed Mar. 16, 1992, now abandoned, which is itself a continuation-in-part of U.S. patent application Ser. No. 07/738,211 filed Jul. 30, 1991, now abandoned, which is itself a continuation-in-part of U.S. patent application Ser. No. 07/670,701 filed Mar. 18, 1991 now U.S. Pat. No. 5,278,299, and U.S. patent application Ser. No. 07/707,600 filed May 30, 1991, now abandoned.

US-CL-CURRENT: 435/252.33, 435/320.1 , 435/69.1 , 435/69.2 , 536/118 , 536/119 , 536/17.2 , 536/17.3 , 536/17.9 , 536/4.1

ABSTRACT:

Oligosacaharide compounds that are substrates and inhibitors of glycosyltransferase and glycosidase enzymes and compositions containing such compounds are disclosed. A method of glycosylation is also disclosed. An E. coli transformed with phagemid CMPSIL-1, which phagemid comprises a gene for a modified CMP-sialic acid synthetase enzyme, which transformed E. coli has the ATCC accession No. 68531 is also provided.

4 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

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US Patent No. - PN (1):
5593887

Detailed Description Text - DETX (241):
These data show that a glycosyl compound can be sialylated in an efficient, enzyme-catalyzed, self-contained, cyclic, synthetic method involving the in situ regeneration of CMP-sialic acid. This synthetic method provides a novel, high-yield (97 percent) scheme for the large-**scale preparation of sialylated** glycosyl compounds.

US-PAT-NO: **5461143**

DOCUMENT-IDENTIFIER: US 5461143 A

TITLE: Oligosaccharide enzyme substrates and inhibitors: methods and compositions

DATE-ISSUED: October 24, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wong; Chi-Huey	San Diego	CA	N/A	N/A
Ichikawa; Yoshitaka	San Diego	CA	N/A	N/A
Shen; Gwo-Jenn	Carlsbad	CA	N/A	N/A

APPL-NO: 08/ 219242

DATE FILED: March 29, 1994

PARENT-CASE:

CROSS-REFERENCE TO COPENDING APPLICATIONS

This application is a continuation of application Ser. No. 07/889,652, filed May 26, 1992 now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 07/852,409, filed Mar. 16, 1992, now abandoned, which is itself a continuation-in-part of U.S. patent application Ser. No. 07/738,211 filed Jul. 30, 1991, now abandoned, which is itself a continuation-in-part of U.S. patent application Ser. No. 07/670,701 filed Mar. 18, 1991, now U.S. Pat. No. 5,278,299, and U.S. patent application Ser. No. 07/707,600 filed May 30, 1991, now abandoned.

US-CL-CURRENT: 536/17.5, 536/118 , 536/119 , 536/17.2 , 536/17.3 , 536/17.9 , 536/4.1

ABSTRACT:

Oligosaccharide compounds that are substrates and inhibitors of glycosyltransferase and glycosidase enzymes and compositions containing such compounds are disclosed. A method of glycosylation is also disclosed. An E. coli transformed with phagemid CMPSIL-1, which phagemid comprises a gene for a modified CMP-sialic acid synthetase enzyme, which transformed E. coli has the ATCC accession No. 68531 is also provided.

11 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

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US Patent No. - PN (1):
5461143

Detailed Description Text - DETX (244):
These data show that a glycosyl compound can be sialylated in an efficient, enzyme-catalyzed, selfcontained, cyclic, synthetic method involving the in situ regeneration of CMP-sialic acid. This synthetic method provides a novel, high-yield (97 percent) scheme for the large-**scale preparation of sialylated** glycosyl compounds.

US-PAT-NO: **5409817**

DOCUMENT-IDENTIFIER: US 5409817 A

TITLE: Use of trans-sialidase and sialyltransferase for synthesis of sialyl.alpha.2.fwdarw.3.beta.galactosides

DATE-ISSUED: April 25, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ito; Yukishige	Tokyo	N/A	N/A	JP
Paulson; James C.	Del Mar	CA	N/A	N/A

APPL-NO: 08/ 057528

DATE FILED: May 4, 1993

US-CL-CURRENT: 435/74, 435/101 , 435/175 , 435/193 , 435/194 , 435/72 , 435/97

ABSTRACT:

A single vessel cyclic synthesis process for preparation of a sialyl.alpha.2.fwdarw.3.beta.galactoside is disclosed. In accordance with this process, a sialyltransferase acceptor is sialylated in an aqueous reaction medium by an .alpha.(2,3)sialyl transferase and CMP-sialic acid to form a sialyl donor substrate and CMP. In the presence of the trans-sialidase of Trypanosoma crusi, that sialyl donor substrate provides a sialyl group for a trans-sialidase acceptor, thereby preparing the sialyl.alpha.2.fwdarw.3.beta.galactoside. The .alpha.(2,3)sialyltransferase acceptor is reformed upon trans-sialidation of the latter acceptor, and the sialyl donor substrate is reformed using the .alpha.(2,3)sialyltransferase and a CMP-sialic acid recycling system that combines CMP with sialic acid that is also present in the vessel. The K.sub.m /V.sub.max value for the .alpha.(2,3)sialyltransferase acceptor is less than one-tenth the value of K.sub.m /V.sub.max of the trans-sialidase acceptor for the .alpha.(2,3)sialyltransferase.

9 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

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US Patent No. - PN (1):

Detailed Description Text - DETX (82):

The results demonstrate the synthetic potential of the T. crusi trans-sialidase. The multienzyme system can be viewed as an extension of the acceptor substrate specificity of sialyltransferases. Due to the broad specificity of the trans-sialidase, many naturally occurring NeuAc.alpha.2.fwdarw.3Gal-OR.sup.2 sequences can be synthesized by substituting different galactoside acceptor substrate. The other advantage of this multienzyme system is that the equilibrium of the trans-sialidase is shifted toward product formation by the sialyltransferase cycle. Because large **scale preparation of both sialyltransferase** and CMP-NeuAc synthetase are now possible [Ichikawa et al., J. Am. Chem. Soc., 114:9283 (1992)], further improvement of efficiency should be possible, once larger scale preparation of a recombinant trans-sialidase is established.

US-PAT-NO: **5278299**

DOCUMENT-IDENTIFIER: US 5278299 A

TITLE: Method and composition for synthesizing sialylated glycosyl compounds

DATE-ISSUED: January 11, 1994

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wong; Chi-Huey	San Diego	CA	N/A	N/A
Ichikawa; Yoshitaka	San Diego	CA	N/A	N/A
Shen; Gwo-Jenn	Carlsbad	CA	N/A	N/A

APPL-NO: 07/ 670701

DATE FILED: March 18, 1991

US-CL-CURRENT: 536/53, 424/94.2 , 424/94.5 , 424/94.61 , 435/193 , 435/194 , 435/200 , 435/84 , 536/124 , 536/26.14 , 536/26.26 , 536/26.8 , 536/4.1 , 536/55.2 , 536/55.3

24 Claims, 3 Drawing figures

Exemplary Claim Number: 1,15,17

Number of Drawing Sheets: 3

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US Patent No. - PN (1):
5278299

Detailed Description Text - DETX (59):
These data show that a glycosyl compound can be sialylated in an efficient, enzyme-catalyzed, self-contained, cyclic, synthetic method involving the in situ regeneration of CMP-sialic acid. This synthetic method provides a novel, high-yield (97 percent) scheme for the large-**scale preparation of sialylated** glycosyl compounds.

Other Reference Publication - OREF (66):
Such a two step synthetic procedure is inadequate, however, for the large **scale preparation of sialylated** glycosyl compounds. The production of activated sialic acid (CMP-sialic acid) is an expensive, time consuming process, which provides CMP-sialic acid only in low yields. Because CMP-sialic acid synthetase

is not commercially available, it must be isolated and purified from animal tissues. About 500 grams of animal tissue are required to produce about 15 Units of the enzyme, an amount sufficient to generate only about 100 .mu.mols of CMP-sialic acid. Higa et al., J. Biol. Chem. 260(15):8838 (1985). Further, the CMP-sialic acid so generated must be isolated and purified prior to use in a transferase reaction. Typically, such isolation and purification procedures involve the separate steps of alcohol extraction, silica-gel chromatography, desalting and analytical chromatography. Higa et al., J. Biol. Chem. 260(15):8838 (1985)..